E-ISSN: 2148-6247

Turkish Journal of PHARMACEUTICAL SCIENCES

An Official Journal of the Turkish Pharmacists' Association, Academy of Pharmacy

Volume: 23 Issue: 1 February 2026



www.turkjps.org

Emerging Sources Citation Index · PubMed Central · PubMed · Scopus · TR Dizin

galenos Publishing House

Turkish Journal of
PHARMACEUTICAL SCIENCES



OWNER

Mehmet İrfan DEMİRCİ on behalf of the Turkish Pharmacists' Association

Editor-in-Chief

Prof. S. Kutay DEMİRKAN, Pharm.D.

Hacettepe University Faculty of Pharmacy Department of Clinical Pharmacy, Ankara, Türkiye
E-mail: ktydmrkn@gmail.com

Associate Editors

Prof. Betül OKUYAN, Ph.D.
Marmara University Faculty of Pharmacy, Department of Clinical Pharmacy, İstanbul, Türkiye
E-mail: betulokuyan@gmail.com

Prof. İ. İrem TATLI ÇANKAYA, Ph.D.
Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Türkiye
E-mail: itatli@hacettepe.edu.tr

Prof. Hande SİPAHİ, Ph.D.
University of Health Sciences Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye
E-mail: handesipahi@hotmail.com

Assoc. Prof. Canan OĞAN HASÇİÇEK, Ph.D.
Ankara University Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye
E-mail: cogan@pharmacy.ankara.edu.tr

Editorial Board

Prof. Afonso Miguel CAVACO, Ph.D.
Lisbon University Faculty of Pharmacy, Department of Pharmacy, Pharmacology and Health Technologies, Lisboa, Portugal
E-mail: acavaco@campus.ul.pt

Prof. Bambang KUSWANDI, Ph.D.
Chemo and Biosensors Group, Faculty of Pharmacy University of Jember, East Java, Indonesia
E-mail: b_kuswandi.farmasi@unej.ac.id

Prof. Bezhan CHANKVETADZE, Ph.D.
Ivane Javakhishvili Tbilisi State University, Institute of Physical and Analytical Chemistry, Tbilisi, Georgia
E-mail: jpba_bezhan@yahoo.com

Prof. Blanca LAFFON, P.D.
DICOMOSA group, Advanced Scientific Research Center (CICA), University of A Coruña, Department of Psychology, Area Psychobiology, Central Services of Research Building (ESCI), Campus Elviña s/n, A Coruña, Spain
E-mail: blanca.laffon@udc.es

Prof. Christine LAFFORGUE, Ph.D.
Paris Saclay University Faculty of Pharmacy, Department of Dermopharmacology and Cosmetology, Paris, France
E-mail: christine.lafforgue@universite-paris-saclay.fr

Prof. Dietmar FUCHS, Ph.D.
Innsbruck Medical University, Center for Chemistry and Biomedicine, Institute of Biological Chemistry, Biocenter, Innsbruck, Austria
E-mail: dietmar.fuchs@i-med.ac.at

Prof. Fernanda BORGES, Ph.D.
Porto University Faculty of Sciences, Department of Chemistry and Biochemistry, Porto, Portugal
E-mail: fborges@fc.up.pt

Prof. Francesco EPIFANO, Ph.D.
Università degli Studi G. d'Annunzio Chieti e Pescara, Chieti CH, Italy
E-mail: francesco.epifano@unich.it

Prof. Göksel ŞENER, Ph.D.
Fenerbahçe University Faculty of Pharmacy, Department of Pharmacology, İstanbul, Türkiye
E-mail: gsener@marmara.edu.tr

Prof. Gülbin ÖZÇELİKAY, Ph.D.
Ankara University Faculty of Pharmacy, Department of Pharmacy Management, Ankara, Türkiye
E-mail: gozcelikay@ankara.edu.tr

Prof. Hermann BOLT, Ph.D.
Dortmund University, Leibniz Research Centre, Institute of Occupational Physiology, Dortmund, Germany
E-mail: bolt@ifado.de

Prof. Hildebert WAGNER, Ph.D.
Ludwig-Maximilians University, Center for Pharmaceutical Research, Institute of Pharmacy, Munich, Germany
E-mail: h.wagner@cup.uni-muenchen.de

Prof. K. Arzum ERDEM GÜRSAN, Ph.D.
Ege University Faculty of Pharmacy, Department of Analytical Chemistry, İzmir, Türkiye
E-mail: arzum.erdem@ege.edu.tr

Prof. Luciano SASO, Ph.D.
Sapienze University Faculty of Pharmacy and Medicine, Department of Physiology and Pharmacology "Vittorio Erspamer", Rome, Italy
E-mail: luciano.saso@uniroma1.it

Prof. Maarten J. POSTMA, Ph.D.
University of Groningen (Netherlands), Department of Pharmacy, Unit of Pharmacoepidemiology and Pharmacoeconomics, Groningen, Holland
E-mail: m.j.postma@rug.nl

Prof. Meriç Köksal AKKOÇ, Ph.D.
Yeditepe University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Türkiye
E-mail: merickoksal@yeditepe.edu.tr

Prof. Robert RAPOPORT, Ph.D.
Cincinati University Faculty of Pharmacy, Department of Pharmacology and Cell Biophysics, Cincinati, USA
E-mail: robertrapoport@gmail.com

Prof. Rob VERPOORTE, Ph.D.
Leiden University, Natural Products Laboratory, Leiden, Netherlands
E-mail: verpoort@chem.leidenuniv.nl

Prof. Tayfun UZBAY, Ph.D.
Üsküdar University Faculty of Medicine, Department of Medical Pharmacology, İstanbul, Türkiye
E-mail: tayfun.uzbay@uskudar.edu.tr

Prof. Wolfgang SADEE, Ph.D.
Ohio State University, Center for Pharmacogenomics, Ohio, USA
E-mail: wolfgang.sadee@osumc.edu

Assoc. Prof. Nadja Cristhina DE SOUZA PINTO, Ph.D.
University of São Paulo, Institute of Chemistry, São Paulo, Brazil
E-mail: nadja@iq.usp.br

Assoc. Prof. Neslihan Aygün KOCABAŞ, Ph.D. E.R.T
Total Research and Technology Feluy Zone Industrielle Feluy, Refining and Chemicals, Strategy-Development-Research, Toxicology Manager, Seneffe, Belgium
E-mail: neslihan.aygun.kocabas@total.com

Turkish Journal of
PHARMACEUTICAL SCIENCES

Advisory Board

Prof. Yusuf ÖZTÜRK, Ph.D.
Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskişehir, Türkiye

Prof. Tayfun UZBAY, Ph.D.
Üsküdar University, Faculty of Medicine, Department of Medical Pharmacology, İstanbul, Türkiye
ORCID: orcid.org/0000-0002-9784-5637

Prof. K. Hüsnü Can BAŞER, Ph.D.
Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye

Prof. Yılmaz ÇAPAN, Ph.D.
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye

Prof. Sibel A. ÖZKAN, Ph.D.
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

Prof. Ekrem SEZİK, Ph.D.
İstanbul Health and Technology University, Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye

Prof. Gönül ŞAHİN, Ph.D.
Eastern Mediterranean University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Famagusta, Cyprus

Prof. Sevda ŞENEL, Ph.D.
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye

Prof. Sevim ROLLAS, Ph.D.
Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Türkiye

Prof. Göksel ŞENER, Ph.D.
Fenerbahçe University, Faculty of Pharmacy, Department of Pharmacology, İstanbul, Türkiye
ORCID: 0000-0001-7444-6193

Prof. Erdal BEDİR, Ph.D.
İzmir Institute of Technology, Department of Bioengineering, İzmir, Türkiye

Prof. Nurşen BAŞARAN, Ph.D.
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye

Prof. Bensu KARAHALİL, Ph.D.
Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye

Prof. Betül DEMİRCİ, Ph.D.
Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye

Prof. Bengi USLU, Ph.D.
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

Prof. Ahmet AYDIN, Ph.D.
Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye

Prof. İlkay ERDOĞAN ORHAN, Ph.D.
Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye

Prof. Ş. Güniz KÜÇÜKGÜZEL, Ph.D.
Fenerbahçe University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Türkiye

Prof. Engin Umut AKKAYA, Ph.D.
Dalian University of Technology, Department of Chemistry, Dalian, CHINA

Prof. Esra AKKOL, Ph.D.
Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye

Prof. Erem BİLENSOY, Ph.D.
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye

Prof. Uğur TAMER, Ph.D.
Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

Prof. Gülaçtı TOPÇU, Ph.D.
Bezmialem Vakıf University, Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye

Prof. Hasan KIRMIZIBEKMEZ, Ph.D.
Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye

Douglas Siqueira DE ALMEIDA CHAVES, Ph.D.
Federal Rural University of Rio de Janeiro, Department of Pharmaceutical Sciences, Rio de Janeiro, Brazil

Members of the Advisory Board consist of the scientists who received Science Award presented by TEB Academy of Pharmacy in chronological order.

Turkish Journal of
PHARMACEUTICAL SCIENCES



Please refer to the journal's webpage (https://www.turkjps.org/) for "Editorial Policy" and "Instructions to Authors".

The editorial and publication process of the **Turkish Journal of Pharmaceutical Sciences** are shaped in accordance with the guidelines of ICMJE, WAME, CSE, COPE, EASE, and NISO. The Turkish Journal of Pharmaceutical Sciences is indexed in **PubMed, PubMed Central, Thomson Reuters / Emerging Sources Citation Index, Scopus, ULAKBİM, Türkiye Citation Index, Embase, EBSCO Host, Türk Medline, Cabi, CNKI.**

The journal is published online.

Owner: Turkish Pharmacists' Association, Academy of Pharmacy

Responsible Manager: Kutay DEMİRKAN

galenos yayınevi

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Türkiye
Phone: +90 (530) 177 30 97
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr | **Publisher Certificate Number:** 14521

Publication Date: July 2026
E-ISSN: 2148-6247
International scientific journal published bimonthly.

Turkish Journal of
PHARMACEUTICAL SCIENCES

CONTENTS

Original Articles

1 **The Effect of Aminoguanidine on Fibrinogen Gene Expression and Oxidative Stress Biomarkers in the Liver Tissue of a Streptozotocin-Induced Diabetic Rat Model**
Amir Karbalaee HASANI, Hosein Fallahi Kord GHESHLAGHI, Mojtaba FATHI, Mina HEMMATI, Vanoushe Azimi PIRSARAEI, Hadi KHODABANDEHLOO

8 **Development of Orally Disintegrating Tablets from Solid Dispersions Containing Tolvaptan/Cyclodextrin Complexes**
Adnan Altuğ KARA, Serdar TORT, Füsun ACARTÜRK

22 **Assessment of the Potential Health Risks Associated with the Toxic and Essential Elements Content in Tea Samples Marketed in Türkiye**
Muhammed HAMİTOĞLU, Mert ŞAHİN, Gülşah ESEN, Sinem HELVACIOĞLU, Ahmet AYDIN

31 **Tannic Acid and Copper-Modified ZIF-8 Metal Organic Framework as a Cefotaxime Delivery System for Antimicrobial Activity**
Yağmur PİRİNÇÇİ TOK, Münteha ÖZSOY, Damla DAMAR ÇELİK

41 **Missed Opportunities in Osteoporosis Prevention and Screening in Postmenopausal Women: Risk Profiles and Awareness**
Fatma ÖZDOĞAN, Burcu KELLECİ ÇAKIR, Şerife Gül ÖZ, Aygin BAYRAKTAR-EKİNCİOĞLU

50 **Pharmacist-Led Ambulatory Blood Pressure Monitoring in Community Pharmacy: An Operational Feasibility Case Series**
Michaela VELLA, Francesca WIRTH, Liberato CAMILLERI, Lilian M. AZZOPARDI

Turk J Pharm Sci 2026;23(1):1-7
DOI: 10.4274/tjps.galenos.2025.48482

ORIGINAL ARTICLE

The Effect of Aminoguanidine on Fibrinogen Gene Expression and Oxidative Stress Biomarkers in the Liver Tissue of a Streptozotocin-Induced Diabetic Rat Model

Amir Karbalaee HASANI[1], Hosein Fallahi Kord GHESHLAGHI[1], Mojtaba FATHI[2], Mina HEMMATI[1], Vanoushe Azimi PIRSARAEI[3], Hadi KHODABANDEHLOO[1]*

[1]Zanjan University of Medical Sciences, Faculty of Medicine, Department of Clinical Biochemistry, Zanjan, Iran
[2]Qazvin University of Medical Sciences, Department of Biochemistry and Genetics, Qazvin, Iran
[3]Zanjan University of Medical Sciences, Faculty of Medicine, Student Research Committee, Zanjan, Iran

ABSTRACT

Objectives: Oxidative stress promotes the initiation and progression of diabetic complications. Fibrinogen is a thrombotic factor proposed as a biomarker for prognostic assessment, as a marker for tracking vascular complications, and as a therapeutic target in diabetes. Aminoguanidine, an inhibitor of advanced glycation end products and inducible nitric oxide synthase, has been shown to reduce oxidative stress and related vascular injury. However, the underlying processes still need to be entirely elucidated. This study investigated the effects of aminoguanidine on oxidative stress biomarkers and fibrinogen gene expression in diabetic rats.

Materials and Methods: Twenty-four male rats, matched for body weight and age, were randomly assigned to five groups: (intended n = 6 per group). Due to mortality during the study, the final group sizes were: healthy controls (n = 5), untreated diabetic controls (n = 4), and diabetic rats treated with aminoguanidine at 50, 100, or 200 mg/kg (n = 6, 4, and 5, respectively). Diabetes mellitus was induced by intraperitoneal injection of streptozotocin (50 mg/kg) in rats fasted for 12 h. One week later, diabetic rats received aminoguanidine for 28 days. The liver was selected for analysis due to its key role in oxidative stress and metabolism. Malondialdehyde (MDA) and ferric-reducing ability of plasma (FRAP) were measured in liver tissue as markers of oxidative stress, and fibrinogen gene expression was assessed by real-time polymerase chain reaction.

Results: Aminoguanidine at 50 mg/kg significantly reduced fibrinogen gene expression ($p = 0.047$). Hepatic MDA levels decreased at all doses (50, 100, and 200 mg/kg, $p < 0.05$), while FRAP levels increased significantly at 50 and 100 mg/kg ($p < 0.05$).

Conclusion: Aminoguanidine may attenuate oxidative stress and fibrinogen gene expression in the livers of diabetic rats.

Keywords: Aminoguanidine, fibrinogen, oxidative stress, diabetes mellitus

INTRODUCTION

Hyperglycemia can cause extensive metabolic changes and increase the risk of thrombosis, potentially leading to myocardial infarction, thrombotic stroke, peripheral vascular disease, and venous thromboembolism.[1,2] Prolonged hyperglycemia correlates with higher levels of advanced glycation end products (AGEs), which increase oxidative stress and thrombotic risk by boosting inflammatory responses.[3,4] Oxidative stress can be assessed using various indices such as malondialdehyde (MDA) and ferric reducing ability of plasma (FRAP).[5,6]

Fibrinogen is a plasma-soluble protein produced by the liver and converted by thrombin into fibrin during hemostasis. Fibrinogen levels are independently associated with glycated hemoglobin levels, suggesting that fibrinogen may increase

*Correspondence: h.khodabandehloo@zums.ac.ir, ORCID-ID: orcid.org/0000-0001-9288-3338
Received: 06.04.2024, Accepted: 23.11.2025 Epub: 09.04.2026 Publication Date: 13.07.2026

Cite this article as: Hasani AK, Gheshlaghi HFK, Fathi M, Hemmati M, Pirsaraei VA, Khodabandehloo H. The effect of aminoguanidine on the fibrinogen gene expression and oxidative stress biomarkers in diabetic rats. Turk J Pharm Sci. 2026;23(1):1-7

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

the risk of cardiovascular events in patients with diabetes. Additionally, AGE levels and fibrinogen have been shown to correlate positively in research,[7-9] suggesting that AGEs and oxidative stress may contribute to thrombosis in diabetic patients.

AGEs can bind to their receptor (RAGE) on hepatocytes, triggering the activation of intracellular signaling pathways, including the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Activation of these pathways promotes the translocation of transcription factors such as NF-κB into the nucleus, where they enhance the transcription of pro-inflammatory and pro-thrombotic genes, including the fibrinogen gene. Oxidative stress further amplifies this process by increasing reactive oxygen species, which can activate similar signaling cascades and stabilize transcription factors, leading to upregulation of fibrinogen expression.[10,11] Evidence indicates that AGEs can increase production of plasminogen activator inhibitor-1, which arrests fibrinolysis by inhibiting tissue plasminogen activator.[12]

Aminoguanidine (AG), a potential therapeutic agent, could prevent the formation of AGEs by reacting with AGE precursors.[13] In light of the role of oxidative stress in thrombotic complications of diabetes, inhibiting the formation of oxidative stress can reduce fibrinogen expression and the incidence of thrombotic events in diabetic patients. Previous studies have reported protective effects of AG in the context of diabetes and its complications. In streptozotocin (STZ)-induced diabetic rats, AG reduced blood glucose levels and partially reversed diabetes-induced alterations in renal function. It also modulated the expression of angiotensin receptors, which may have contributed to its protective effects on hemodynamic and physiological functions.[14] Another study demonstrated that AG prevented cardiac fibrosis and the development of diabetic cardiomyopathy.[15] In addition, an *in vitro* study using beta-cells showed that exposure to high glucose concentrations for one week significantly reduced insulin secretion and insulin content. Co-treatment with 1 mM AG nearly doubled both basal and glucose-stimulated insulin release, and increased insulin content by 42%, and reduced nitrite accumulation by 33%. These findings suggest that AG may protect beta-cells against glucotoxicity, potentially through modulation of early glycation products and nitric oxide synthase activity.[16] Despite these promising results, some limitations have been noted, including variability in experimental models and dose-dependent effects, which warrant further investigation before translation into clinical practice.

Understanding the effects of AG on oxidative stress and fibrinogen expression is crucial, as it may offer a therapeutic strategy for reducing thrombotic complications in diabetes and provide a rationale for future clinical research. This study assessed the impact of AG on fibrinogen gene expression and on MDA and FRAP levels in the livers of STZ-induced diabetic rats.

MATERIALS AND METHODS

Animal models and study design

The study was conducted on adult male Rattus norvegicus provided by the Experimental Animal Research Center of Zanjan University of Medical Sciences (Zanjan, Iran). The rats (weighing 250–300 g) were kept at room temperature (22–25 °C) with a 12-hour light–dark cycle and unrestricted access to standard laboratory chow (ParsCenter, Iran) and water. Body weight and blood glucose levels were monitored every seven days during the experimental period.

The STZ-induced diabetic rat model was selected because STZ selectively destroys pancreatic beta-cells, reliably inducing hyperglycemia and closely mimicking type 1 diabetes and its associated metabolic disturbances. The liver was chosen as the target organ due to its central role in glucose and lipid metabolism and its susceptibility to diabetes-induced oxidative stress and inflammation. Focusing on the liver allowed assessment of both metabolic and molecular effects of AG in a key organ affected by diabetic complications.[17,18]

The sample size for each group was determined by a priori power analysis (G*Power) with $\alpha = 0.05$ and 80% power, indicating that 4–6 rats per group were required. A total of 24 rats, matched for age and body weight, were randomly assigned to five groups, with an intended size of 6 rats per group. Due to mortality during the study, the final group sizes were: 5 healthy rats on a standard diet (HC group); 4 untreated diabetic rats on a standard diet (DC group); 6 diabetic rats receiving 50 mg/kg of AG (D50 group); 4 diabetic rats receiving 100 mg/kg of AG (D100 group); and 5 diabetic rats receiving 200 mg/kg of AG (D200 group).[19] Randomization was performed using a random number table to minimize allocation bias, and group assignment was conducted by a researcher who was not involved in subsequent interventions or outcome assessments. In addition, the experiments and data analyses were performed by another researcher who was blinded to the group assignments, to further reduce potential bias.

An intraperitoneal injection of 50 mg/kg STZ, dissolved in 0.9% saline, was used to induce diabetes mellitus in rats fasted for 12 hours. Seventy-two hours after the STZ injection, blood glucose levels in fasted rats were measured from tail blood samples using the Accu-Chek Aviva Blood Glucose Monitoring device. Rats classified as diabetics had fasting blood glucose levels above 250 mg/dL. Treatment with intraperitoneal AG (Sigma-Aldrich Co., Cat No. 1068-42-4) began one week after diabetes induction and continued daily for four weeks.[19] Both healthy and untreated diabetic rats received daily saline as a placebo (Table 1). Approval for all procedures conducted was obtained from the Animal Care and Use Committee of Zanjan University of Medical Sciences (approval number: IR.ZUMS.REC.1398.434, dated 14.01.2020).

Liver tissue collection

Ketamine and xylazine were administered intraperitoneally to the rats at the end of the experimental period to induce anesthesia. After removal of the liver, it was rinsed with an

Table 1. Experimental design of the study: animal groups, induction method, and treatments.

Groups	Number of rats	Condition	Induction method	Treatment	Duration
HC	5	Healthy	—	Saline (placebo)	4 weeks
DC	4	Diabetic	STZ (50 mg/kg, i.p.)	Saline (placebo)	4 weeks
D50	6	Diabetic	STZ (50 mg/kg, i.p.)	AG 50 mg/kg (i.p.)	4 weeks
D100	4	Diabetic	STZ (50 mg/kg, i.p.)	AG 100 mg/kg (i.p.)	4 weeks
D200	5	Diabetic	STZ (50 mg/kg, i.p.)	AG 200 mg/kg (i.p.)	4 weeks

AG, aminoguanidine; DC, diabetic control; HC, healthy control; i.p., intraperitoneal; STZ, streptozotocin.

isotonic saline solution and weighed. The tissue was divided into two sections: (a) the first was stored at -80 ˚C for RNA and molecular analyses; (b) the second was homogenized for antioxidant analysis.

FRAP assay

FRAP was measured in the liver using the methods of Benzie and Strain.[20] The intra-assay and inter-assay coefficients of variation were < 1.0% and < 3.0%, respectively, for FRAP values between 100 and 1,000 µmol/L of protein. The FRAP reagent was prepared by incubating a mixture of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl, 20 mmol/L ferric chloride hexahydrate (FeCl3·6H2O), and 0.3 mol/L acetate buffer (pH 3.6) at a 1:1:10 (*v/v/v*) ratio at 37 ˚C for 10 minutes.

The technique was based on converting the Fe3+-TPTZ complex to the Fe2+-TPTZ complex at low pH, and monitoring the process by detecting the change in absorbance at 593 nm. 300 µL of prepared FRAP reagent and 30 µL of H2O were mixed with 10 µL of the sample solution, incubated at 37 ˚C for 5 minutes, and absorbance was measured using a spectrophotometer. Calibration was conducted using FeSO4·7H2O solutions within the range of 100–1000 µmol/L, and the FRAP value in the tissue was reported as µmol/L of protein.

MDA assay

Lipid peroxidation was assessed by reaction of MDA with thiobarbituric acid according to the methodology outlined by Ohkawa et al.[21] The reaction mixture included 1.5 mL of a 20% acetic acid solution (pH 3.5), 0.1 mL of supernatant, 0.2 mL of sodium dodecyl sulfate (8.1%), and 1.5 mL of a 0.8% aqueous thiobarbituric acid solution. After adding 4 mL of distilled water, the mixture was heated at 95 ˚C for 60 minutes. Distilled water (5 mL) and an n-butanol-pyridine mixture (15:1 *v/v*, 5 mL) were added to the mixture, and it was centrifuged at 4000 r.p.m. for 10 minutes. In the blank, the supernatant was replaced with distilled water. Finally, the results were expressed as µmol L–1 using a spectrophotometer to measure the absorbance of the top layer at 532 nm.

RNA isolation and Complementary DNA (cDNA) synthesis

The RNX-Plus kit (SinaClon Co., Tehran, Iran) was used to isolate total RNA from liver tissue. Briefly, 200 µL of chloroform was added to the homogenized liver tissue in 1 mL Trizol, and the mixture was centrifuged at 12,000 r.p.m. and 4 ˚C for 15 minutes. The top aqueous phase was transferred to a new RNase-free tube, combined with an equal volume of isopropanol, vortexed vigorously, and centrifuged at 12,000 rpm at 4 ˚C for 15 minutes. The supernatants were discarded; 1 mL of 75% ethanol was added, and the RNA pellets were centrifuged at 7,500 r.p.m. and 4 ˚C for 8 minutes. After discarding the supernatants and air-drying at room temperature for a few minutes, the pellets were resuspended in 50 µL of DEPC-treated water.

Agarose gel electrophoresis and UV absorbance measurements at 230, 260, and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) were used to assess RNA sample quality and quantity.

cDNA was generated from total RNA using a cDNA synthesis kit (BioFact™ Co., Korea) according to the manufacturer's protocol. After purified total RNA (5 µg) was incubated with oligo dT (1 µL) for 5 minutes at 70˚C, the following components were added to the mixture: 4 µL of 5X reaction buffer, 1 µL of dNTPs (10 mM each), 0.5 µL of RNase inhibitor (40 U/µL), and 1 µL of reverse transcriptase (200 U/µL). The reaction was then incubated at 42 ˚C for 60 minutes, followed by 5 minutes at 70 ˚C.

Real-time polymerase chain reaction (PCR)

Synthesized cDNA (1 µL) was mixed with forward and reverse primers (0.5 µL each), deionized water, and SYBR Green I master mix. The ABI StepOnePlus real-time PCR system was used to perform real-time PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the housekeeping gene due to its widespread use and reported stability in gene expression studies.[22] Ct-value analysis across all experimental groups confirmed that GAPDH expression remained stable under our experimental conditions; therefore, it was used to normalize fibrinogen expression levels.

To ensure the accuracy of the real-time PCR results, both no template control (NTC) and no reverse transcriptase (NRT) reactions were included. The NTC contained all PCR components except cDNA, which was replaced with water; any amplification in this control indicated potential environmental contamination or non-specific priming. The NRT control contained all reaction components; NRT products, generated alongside cDNA synthesis, were added in place of cDNA. Amplification in this control indicated possible contamination with genomic DNA during RNA extraction. NTC reactions were performed in triplicate alongside biological replicates. These procedures ensured that PCR results were not affected by contamination or non-specific amplification.

The PCR cycling conditions involved a 15-minute initial activation step at 95 ˚C followed by 40 cycles at 95 ˚C for 15 seconds. The final temperatures were 60 ˚C and 72 ˚C, each for 30 seconds. Table 2 displays the primer sequences used in this research. The fold change was calculated using the 2-ΔCt method. To verify product quality, aliquots of real-time PCR reactions that had been dye-stained and subjected to agarose gel electrophoresis were visualized using a UV transilluminator.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 24.0. The normality of the data was assessed using the Kolmogorov–Smirnov test. The means of two or more independent groups were compared using one-way analysis of variance. GraphPad Prism version 8.0 (GraphPad Software, Inc., CA, USA) was used to generate graphs, and statistical significance was defined as a *p*-value of 0.05 or less. Outliers were identified as values exceeding ±3 standard deviations from the group mean and were excluded from the analysis. No missing data were encountered in this study.

RESULTS

The fibrinogen gene expression

The results demonstrated that the level of fibrinogen gene expression in the liver of untreated diabetic rats was significantly higher than that in healthy rats ($p = 0.001$). Figure 1 shows the comparison of fibrinogen gene expression between the diabetic and healthy groups. In this study, a 28-day treatment with AG at doses of 50, 100, and 200 mg/kg/day decreased the expression of the fibrinogen gene. However, this reduction was statistically significant only at 50 mg/kg/day ($p = 0.047$). Fibrinogen gene expression in treated diabetic rats compared with untreated diabetic rats is presented in Figure 1.

The levels of MDA

Our results suggested that MDA levels in untreated diabetic rats were higher than those in healthy rats ($p < 0.05$). The MDA levels among the untreated rats are illustrated in Figure 2. Measurement of MDA levels, as a marker of oxidative stress, demonstrated that all three doses of AG reduced MDA levels in the livers of diabetic rats ($p < 0.001$). The MDA levels after 28 days of treatment with various doses of AG are shown in Figure 2.

The levels of FRAP

FRAP measurements indicated that the untreated diabetic rats had lower FRAP levels than the healthy group ($p < 0.05$). The FRAP levels in the liver tissue of untreated rats are shown in Figure 3. AG injections increased FRAP levels in diabetic rats, with significant increases at doses of 50 and 100 mg/kg/day compared with untreated diabetic rats ($p < 0.05$). The FRAP levels after treatment with different doses of AG are shown in Figure 3.

Table 2. Detailed information regarding the primers utilized in the study.

Gene	Primer sequence (5' to 3')	Length of target
Fibrinogen	Forward: CAAAGGGCAACTGCTTGGAAT Reverse: CGGAGTCAGTAGTTGCAGCTT	132 bp
GAPDH	Forward: CAACTCCCTCAAGATTGTCAGCAA Reverse: GGCATGGACTGTGGTCATGA	118 bp

Bp, base pair; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

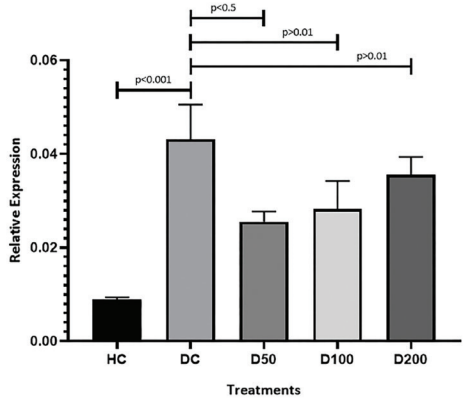


Figure 1. Comparison of fibrinogen gene expression between healthy and diabetic groups and after treatment with aminoguanidine. The data were reported as 2-ΔCt.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; HC, healthy control.

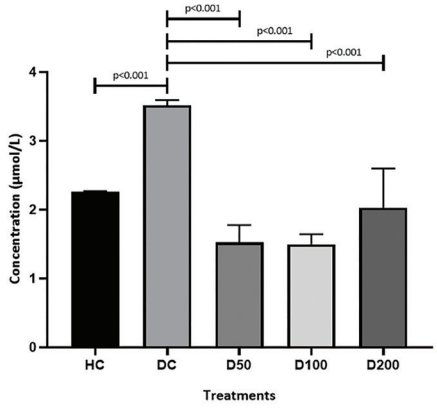


Figure 2. Comparison of malondialdehyde levels between healthy and diabetic groups and after treatment with aminoguanidine. Data were presented as mean ± standard deviation.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; HC, healthy control.

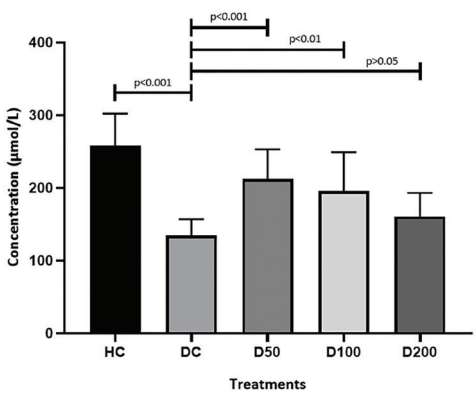


Figure 3. Comparison of FRAP levels between healthy and diabetic groups and after treatment with aminoguanidine. Data were presented as mean ± standard deviation.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; FRAP, ferric reducing antioxidant of plasma; HC, healthy control.

DISCUSSION

Cardiovascular diseases account for the majority of deaths among individuals with diabetes mellitus; diabetes is associated with a two- to threefold greater risk of cardiovascular diseases. Evidence suggests that excessive oxidative stress may play a vital role in the development of both diabetes and cardiovascular diseases. Several studies have shown that plasma markers of oxidative stress are elevated in individuals with coronary artery disease or its risk factors.

The present study demonstrated that STZ-induced diabetes mellitus led to oxidative stress and altered MDA and FRAP levels in the livers of diabetic rats. STZ, a toxic glucose analog, selectively damages pancreatic beta cells, leading to oxidative stress.[23] Elevated MDA levels during oxidative stress indicate lipid peroxidation. Preventing oxidative stress can help reduce the severity of diabetes mellitus. Thus, it is crucial to avoid the generation of free radicals by diabetes medications.

The current study assessed the impact of AG on oxidative stress and fibrinogen gene expression in diabetes mellitus. Mechanistically, AG may influence fibrinogen gene expression by modulating oxidative stress–related signaling pathways. Increased oxidative stress in diabetes can activate transcription factors such as NF-κB, which upregulate fibrinogen expression. By reducing reactive oxygen species and enhancing antioxidant defenses, AG may inhibit NF-κB activation, thereby downregulating fibrinogen transcription. Additionally, AG might interact with other signaling pathways involved in inflammation and coagulation, such as the MAPK and JAK/STAT pathways, contributing to its regulatory effect on fibrinogen gene expression.[10,11]

AG contains a nucleophilic hydrazine and a guanidine group, both of which can react with dicarbonyl compounds and biological molecules such as pyridoxal phosphate, pyruvate, glucose, and MDA. AG has been shown to have both antioxidant and pro-oxidant effects.[24]

MDA measurements revealed that all three AG doses significantly reduced MDA levels in STZ-induced diabetic rats. In addition, AG at doses of 50 and 100 mg/kg notably elevated FRAP levels in the livers of diabetic rats. Consistent with these findings, Rastegar-Moghaddam et al.[25] demonstrated that AG administration attenuated hypothyroidism-induced liver injury by significantly lowering MDA, improving liver function tests, restoring antioxidant enzyme activities, and reducing fibrotic changes, particularly at a dose of 30 mg/kg. Similarly, in a model of renal ischemia/reperfusion injury, co-administration of AG mitigated the adverse effects of sumatriptan by reducing serum creatinine and blood urea nitrogen levels, decreasing MDA, and restoring superoxide dismutase activity.[26] In an alloxan-induced rabbit model of type 1 diabetes, AG administration significantly decreased early glycation products, including HbA1c and fructosamine, restored the activity of key antioxidant enzymes, and reduced excessive nitric oxide production.[27] Moreover, Hosseini et al.[28] demonstrated that AG exerted protective effects against endotoxin-induced renal dysfunction in rats, evidenced by reductions in MDA and nitric oxide metabolites, along with restored activities of superoxide dismutase and catalase. Collectively, these studies indicate that the protective effects of AG are largely mediated by inhibition of nitric oxide production from inducible nitric oxide synthase and enhancement of endogenous antioxidant and anti-inflammatory defenses, thereby alleviating oxidative and nitrosative stress-related tissue damage.

Other studies used *in vitro* models of oxidized bovine serum albumin with glucose as a glycating agent to assess antioxidant activity and found that the group treated with AG had higher FRAP levels than the untreated group.[29,30] The FRAP levels in the colon of the irritable bowel syndrome model were not significantly different from those in the AG-treated group (100 mg/kg, intraperitoneally).[31] The models examined in the studies mentioned above could account for the disparity in results. Overall, these findings indicate that the antioxidant effects of AG, demonstrated both *in vitro* and in animal models, may have clinical relevance by helping to mitigate oxidative stress in diabetes and thereby reduce the risk of cardiovascular complications.

The role of fibrinogen in the pathogenesis of atherosclerosis, and its consequences in patients with diabetes mellitus, has been investigated; previous research has confirmed the vital role of fibrinogen in the development of cardiovascular events.[32,33] In addition to modulating oxidative stress and fibrinogen expression, AG may confer cardiovascular protection by improving endothelial function, reducing fibrin thrombus formation, and regulating fibrinolytic factors such as plasminogen activator inhibitor-1 and tissue-type plasminogen activator.[34] However, investigations on the effects of AG on fibrinogen gene expression in diabetes mellitus were limited.

The current study demonstrated that treatment with AG at various doses reduced fibrinogen gene expression in diabetic rats. This reduction was statistically significant only in the 50 mg/kg/day dose. This dose–response relationship suggested

that moderate dosing was optimal for modulating fibrinogen expression, potentially reflecting a balance between inhibition of AGEs and avoidance of compensatory cellular responses that occur at higher doses. Further studies are needed to clarify the underlying molecular mechanisms and to determine the most effective dose range of AG for preventing vascular complications in diabetes.

Su et al.[35] found that plasma fibrinogen levels were higher in patients with cardiovascular disease who died than in participants who survived. No formation of fibrin thrombi was observed in the livers of pigs in the AG group, either before or at the end of the ischemic period. However, the AG group showed reduced fibrin thrombus formation after reperfusion compared with other groups.[36] In another study, treatment with AG (25 mmol/L) during glycation of low-density lipoprotein cholesterol significantly decreased plasminogen activator inhibitor-1 and increased tissue-type plasminogen activator in vascular endothelial cells.[37]

Based on the findings of the current study, future research could explore the long-term effects of AG on oxidative stress and fibrinogen expression in diabetic models. Studies in different animal models, including larger mammals, would help to confirm the generalizability of these results. Furthermore, clinical trials could evaluate the potential translational benefits of AG in managing oxidative stress and reducing cardiovascular risk in patients with diabetes. Additional investigations into the underlying molecular mechanisms and optimal dosing strategies are warranted to maximize therapeutic efficacy and safety.

Study limitations

This study had several limitations that should be noted. First, the sample size was relatively small, which may limit the statistical power and generalizability of the findings. Second, the study duration was short, and the longer-term effects of AG on oxidative stress and fibrinogen expression remain unclear. Third, the use of the STZ-induced diabetic rat model, while widely accepted, may not fully replicate all aspects of human diabetes and its cardiovascular complications. Finally, the study did not assess the potential side effects or toxicity of AG, which should be considered in future investigations. Future studies addressing these limitations would strengthen the evidence base for the therapeutic potential of AG.

CONCLUSION

Diabetes mellitus is a complex metabolic disease in which oxidative stress and hyperfibrinogenemia play central roles in disease progression and the development of cardiovascular complications. In this study, AG, an inhibitor of AGE formation, significantly reduced oxidative damage and fibrinogen gene expression in diabetic rats. These findings suggest that AG may have clinical benefits in mitigating oxidative stress, regulating fibrinogen levels, and lowering cardiovascular risk in patients with diabetes. Future studies are warranted to optimize dosing, evaluate long-term effects, and confirm these protective effects in clinical settings.

Ethics

Ethics Committee Approval: Approval for all procedures conducted was obtained from the Animal Care and Use Committee of Zanjan University of Medical Sciences (approval number: IR.ZUMS.REC.1398.434, dated 14.01.2020).

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Design: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Data Collection or Processing: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Analysis or Interpretation: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Literature Search: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Writing: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Li X, Weber NC, Cohn DM, Hollmann MW, DeVries JH, Hermanides J, Preckel B. Effects of hyperglycemia and diabetes mellitus on coagulation and hemostasis. J Clin Med. 2021;10:2419.
2. Kwaifa IK, Bahari H, Yong YK, Noor SM. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. Biomolecules. 2020;10:291.
3. Fuhr JC, Ramos MEK, Piovesan F, Renner LdO, Siqueira LdO. Relationship of advanced glycation end-products in hypertension in diabetic patients: a systematic review. J Bras Nefrol. 2022;44:557-572.
4. Chen Y, Meng Z, Li Y, Liu S, Hu P, Luo E. Advanced glycation end products and reactive oxygen species: uncovering the potential role of ferroptosis in diabetic complications. Mol Med. 2024;30:141.
5. Alam R, Ahsan H, Khan S. The role of malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP) in patients with hypertension. Molecular and Cellular Biomedical Sciences. 2023;7:58-64.
6. Cordiano R, Di Gioacchino M, Mangifesta R, Panzera C, Gangemi S, Minciullo PL. Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: an update. Molecules. 2023;28:5979.
7. Vilar R, Fish RJ, Casini A, Neerman-Arbez M. Fibrin(ogen) in human disease: both friend and foe. Haematologica. 2020;105:284.
8. Abdelmonem M, Abdulla S, Wasim H, Elawamy H. The correlation between plasma fibrinogen levels and glycemic status in type 2 diabetes mellitus. Am J Clin Pathol. 2023;160(Supplement 1):S19-S20.
9. Luzak B, Boncler M, Kosmalski M, Mnich E, Stanczyk L, Przygodzki T, Watala C. Fibrinogen glycation and presence of glucose impair fibrin polymerization—an *in vitro* study of isolated fibrinogen and plasma from patients with diabetes mellitus. Biomolecules. 2020;10:877.
10. Pathomthongtaweechai N, Chutipongtanate S. AGE/RAGE signaling-mediated endoplasmic reticulum stress and future prospects in non-coding RNA therapeutics for diabetic nephropathy. Biomed Pharmacother. 2020;131:110655.
11. Zhang Y, Zhang Z, Tu C, Chen X, He R. Advanced glycation end products in disease development and potential interventions. Antioxidants. 2025;14:492.

12. Khalid M, Petroianu G, Adem A. Advanced glycation end products and diabetes mellitus: mechanisms and perspectives. Biomolecules. 2022;12:542.

13. Song Q, Liu J, Dong L, Wang X, Zhang X. Novel advances in inhibiting advanced glycation end product formation using natural compounds. Biomed Pharmacother. 2021;140:111750.

14. Jogula RMR, Row AT, Siddiqui AH, Row A. The effect of treatment with aminoguanidine, an advanced glycation end product inhibitor, on streptozotocin-induced diabetic rats and its effects on physiological and renal functions. Cureus. 2023;15.

15. Heydari AH, Fathi M, Heydari S, Heidari ME. Advanced glycation end product blocker drugs have a great potential to prevent diabetic cardiomyopathy in an animal model of diabetes mellitus type 2. Cardiovasc Ther. 2022;2022:7014680.

16. Tajiri Y, Grill V. Aminoguanidine exerts a β-cell function-preserving effect in high glucose-cultured β-cells (INS-1). J Diabetes Res. 2000;1:111-119.

17. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 2023;22:274.

18. Vesković M, Šutulović N, Hrnčić D, Stanojlović O, Macut D, Mladenović D. The interconnection between hepatic insulin resistance and metabolic dysfunction-associated steatotic liver disease—the transition from an adipocentric to liver-centric approach. Curr Issues Mol Biol. 2023;45:9084-9102.

19. Alipour M, Adineh F, Mosatafavi H, Aminabadi A, Monirinasab H, Jafari MR. Effect of chronic intraperitoneal aminoguanidine on memory and expression of Bcl-2 family genes in diabetic rats. Can J Physiol Pharmacol. 2016;94:669-675.

20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-76.

21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-358.

22. Shahsavarpour S, Shafiei M, Buratti E, Galehdari H. Identification of reliable housekeeping genes for qRT-PCR normalization in neuroblastoma and glioblastoma cell lines infected with lentivirus. Gene, Cell and Tissue. 2025;12.

23. Salazar-García M, Corona JC. The use of natural compounds as a strategy to counteract oxidative stress in animal models of diabetes mellitus. Int J Mol Sci. 2021;22:7009.

24. Köhler ZM, Szepesi Á. More than a diamine oxidase inhibitor: L-aminoguanidine modulates polyamine-related abiotic stress responses of plants. Life. 2023;13:747.

25. Rastegar-Moghaddam SH, Kiumarsi Z, Mirani A, Mortazavi M, Beheshti F, Ebrahimzadeh-Bideskan A, Anaeigoudari A, Hosseini M. Aminoguanidine improved liver function and attenuated oxidative stress in hypothyroid rats by propylthiouracil. Adv Biomed Res. 2025;14:64.

26. Mobasheran P, Rajai N, Kohansal P, Dehpour AR, Shafaroodi H. The effects of acute sumatriptan treatment on renal ischemia/reperfusion injury in rat and the possible involvement of nitric oxide. Can J Physiol Pharmacol. 2020;98:252-258.

27. Arif B, Arif Z, Ahmad J, Perveen K, Bukhari NA, Ashraf JM, Rabbani G. Attenuation of hyperglycemia and amadori products by aminoguanidine in alloxan-diabetic rabbits occurs via enhancement in antioxidant defenses and control of stress. PLoS One. 2022;17:e0262233.

28. Hosseini M, Beheshti F, Anaeigoudari A. Improving effect of aminoguanidine on lipopolysaccharide-caused kidney dysfunction in rats. Saudi J Kidney Dis Transpl. 2020;31:1025-1033.

29. Pawlukianiec C, Gryciuk ME, Mil KM, Żendzian-Piotrowska M, Zalewska A, Maciejczyk M. A new insight into meloxicam: assessment of antioxidant and anti-glycating activity in *in vitro* studies. Pharmaceuticals. 2020;13:240.

30. Mil KM, Gryciuk ME, Pawlukianiec C, Żendzian-Piotrowska M, Ładny JR, Zalewska A, Maciejczyk M. Pleiotropic properties of valsartan: do they result from the antiglycooxidant activity? Literature review and *in vitro* study. Oxid Med Cell Longev. 2021;2021:1-14.

31. Dolatabadi F, Abdolghaffari AH, Farzaei MH, Baeeri M, Ziarani FS, Eslami M, Abdollahi M, Rahimi R. The protective effect of Melissa officinalis L. in visceral hypersensitivity in rat using two models of acid-induced colitis and stress-induced irritable bowel syndrome: a possible role of nitric oxide pathway. J Neurogastroenterol Motil. 2018;24:490.

32. Surma S, Banach M. Fibrinogen and atherosclerotic cardiovascular diseases—review of the literature and clinical studies. Int J Mol Sci. 2021;23:193.

33. Sun J, Han K, Xu M, Li L, Qian J, Li L, Guo Y, Chen Y. Blood viscosity in subjects with type 2 diabetes mellitus: roles of hyperglycemia and elevated plasma fibrinogen. Front Physiol. 2022;13:827428.

34. Beheshti F, Hosseini M, Hashemzehi M, Hadipanah MR, Mahmoudabady M. The cardioprotective effects of aminoguanidine on lipopolysaccharide-induced inflammation in rats. Cardiovasc Toxicol. 2020;20:474-481.

35. Su H, Cao Y, Chen Q, Ye T, Cui C, Chen X, Zhang Y, Lin X, Zhou Y. The association between fibrinogen levels and severity of coronary artery disease and long-term prognosis following percutaneous coronary intervention in patients with type 2 diabetes mellitus. Front Endocrinol. 2023;14:1287855.

36. Isobe M, Katsuramaki T, Hirata K, Kimura H, Nagayama M, Matsuno T. Beneficial effects of inducible nitric oxide synthase inhibitor on reperfusion injury in the pig liver. Transplantation. 1999;68:803-813.

37. Zhang J, Ren S, Sun D, Shen GX. Influence of glycation on LDL-induced generation of fibrinolytic regulators in vascular endothelial cells. Arterioscler Thromb Vasc Biol. 1998;18:1140-1148.

Turk J Pharm Sci 2026;23(1):8-21
DOI: 10.4274/tjps.galenos.2026.20666

ORIGINAL ARTICLE

Development of Orally Disintegrating Tablets from Solid Dispersions Containing Tolvaptan/ Cyclodextrin Complexes

Adnan Altuğ KARA[1], Serdar TORT[2], Füsun ACARTÜRK[2]*

[1]Başkent University Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye
[2]Gazi University Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Türkiye

ABSTRACT

Objectives: Tolvaptan is a compound that is practically insoluble in water and poorly soluble at physiological pH, and is used to treat low blood sodium levels in adults with conditions such as heart failure and certain hormonal imbalances. Increasing the water solubility and dissolution rate of tolvaptan could increase its bioavailability and, consequently, its efficacy. This study aimed to increase the efficiency of tolvaptan by enhancing its solubility and dissolution rate, and to develop a fast-acting dosage form that improves convenience for patients with swallowing difficulties.

Materials and Methods: Solid dispersion (SD) formulations were developed by the rotary evaporation method using hydrophilic polymers (polyvinylpyrrolidone and polyethylene oxide), solubility enhancers (Solutol HS-15 and Gelucire 44/14), and complexing agents (β-cyclodextrin and hydroxypropyl-β-cyclodextrin). Various characterization studies were performed on developed formulations, including solubility studies, X-ray diffraction, differential scanning calorimetry, Fourier transform infrared spectroscopy, and scanning electron microscopy analyses, *in vitro* dissolution tests, and release kinetics studies.

Results: The solubility of tolvaptan in the 2-hydroxypropyl-beta-cyclodextrin (HPβCD)-SD2 formulation containing HPβCD, was the highest at 0.2314 mg/mL, which was approximately 8.7 times that of pure tolvaptan. Based on the results, HPβCD was selected as the complexing agent as the optimal SD formulation. In the dissolution study, at least 90% of the tolvaptan in the HPβCD-SD2 formulation dissolved in all buffer solutions within 25 min. Orally disintegrating tablets (ODTs) were prepared using the HPβCD-SD2 formulation; formulation code 59, which had a friability of ≤1% and a disintegration time of ≤180 s, was selected as the final tablet.

Conclusion: A new SD formulation with increased solubility and dissolution rate compared with pure tolvaptan was developed. The developed ODTs may be an alternative to the current commercial product.

Keywords: Solid dispersion(s), orally disintegrating tablet, tolvaptan, cyclodextrin, solubility and dissolution rate

***Correspondence:** acarturk@gazi.edu.tr, **ORCID-ID:** orcid.org/0000-0001-9515-750X
Received: 17.08.2025, **Accepted:** 11.02.2026 **Epub:** 15.04.2026 **Publication Date:** 13.07.2026

Cite this article as: Kara AA, Tort S, Acartürk F. Development of orally disintegrating tablets from solid dispersions containing tolvaptan/cyclodextrin complexes. Turk J Pharm Sci. 2026;23(1):8-21

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

INTRODUCTION

Solid dispersion (SD) is an important formulation development strategy defined as the dispersion of one or more active substances in a solid, inert carrier or matrix prepared by solvent evaporation, melting, or solvent-melting.[1–3] In the solvent evaporation method, an SD is obtained after the solvent evaporates from the solution containing the drug and carrier; the resulting solid mass is then pulverized and milled.[4,5] In this method, heat degradation of drugs or carriers can be prevented because organic solvents evaporate at low temperatures.[5,6] Methods such as hot-plate heating, vacuum drying, rotary evaporation, spray drying, freeze drying, spray-freeze drying, and ultra-fast freezing are used to remove the solvent quickly.[7] The rotary evaporation method is a solvent evaporation technique that involves evaporating a dispersion or solution under vacuum in a rotating flask for a specified period, resulting in the formation of a solid film on the inner glass surface of the flask when it is heated. This method is rapid, relatively inexpensive, and readily available.

Cyclodextrins (CDs) can form inclusion complexes with active substances that have low solubility and dissolution rates, thereby improving their properties owing to their hydrophilic outer surfaces.[8,9] In terms of formulation development, CDs offer advantages, such as reducing particle size to the molecular level, increasing wettability and porosity, and converting the drug's crystalline state into an amorphous form.[7,10,11] The aim of a study was to increase the oral bioavailability of sulfamethoxazole, which has low solubility and a low dissolution rate, by enhancing its solubility and dissolution rate. For this purpose, 2-hydroxypropyl-beta-cyclodextrin (HPβCD): sulfamethoxazole (1:1) complexes were prepared, and SDs were prepared using polyethylene glycol 20000 and polysorbate 20. The solubility of sulfamethoxazole was increased by forming complexes with CDs and by preparing SDs of these complexes.[12]

Although tablets are widely used today, many elderly individuals and children may have difficulty swallowing them. Orally disintegrating tablet (ODT) formulations are being developed for these patients. The Food and Drug Administration (FDA) defines an ODT as a solid dosage form that disperses rapidly, usually within a few seconds, when placed on the tongue, and contains a medicinal substance or an active ingredient.[13] According to the European Pharmacopoeia, ODTs disperse *in vitro* in water within 3 min.[14] ODTs have many advantages, such as applicability to patients who have difficulty swallowing or who refuse to swallow, rapid drug effects, no water requirement, improved stability, and increased bioavailability.[15] In general, excipients such as fillers, superdisintegrants, glidant-lubricants, sweeteners, and salivary-stimulating agents are used in ODT formulations.[16] In a study, orally disintegrating risperidone tablets were developed using SD and CD technologies. An SD of risperidone was prepared using methyl-CD; an increased dissolution rate was observed. They found that the disintegration time of SD ODTs containing risperidone prepared with Ac-Di-Sol and mannitol was 22.83 ± 3.66 s.[17]

Hyponatremia can occur frequently in older adults, especially in those who are hospitalized or living in long-term care facilities,[18] as well as diarrhea that occurs in childhood.[19] Tolvaptan, a vasopressin V2 receptor antagonist, is used to treat hyponatremia by increasing low blood sodium levels in adults with conditions such as heart failure and certain hormonal imbalances, and it plays a role in regulating renal fluid excretion.[20,21] Tolvaptan is a BCS 4 drug that is practically insoluble in water, with low solubility at all pH values.[22] It is important to improve its efficacy by enhancing the solubility and dissolution rate in water. In a study, SDs of tolvaptan were prepared by hot-melt extrusion and spray-drying techniques using hydrophilic polymers such as Soluplus, Kollidon VA64, and Kollidon 30. In the solubility study conducted in distilled water, the solubilities of pure tolvaptan, tolvaptan in SD prepared by spray drying, and tolvaptan in SD prepared by hot-melt extrusion were 0.04 ± 0.02, 0.32 ± 0.10, and 0.29 ± 0.05 mg/mL, respectively. In the *in vitro* dissolution test performed in distilled water containing 0.22% sodium lauryl sulfate (SLS), 39.60% of pure tolvaptan, 93.50% of tolvaptan in SDs obtained by hot-melt extrusion method, 91.40% of tolvaptan in SDs obtained by spray drying, and 94.20% of tolvaptan in the commercial product were released within 60 min.[23] In another study, conventional ODTs containing pure tolvaptan and superdisintegrants were prepared by wet granulation. As a result of the study, the disintegration time ranged from 40 ± 0.01 to 105 ± 0.01 s, and friability ranged from 0.64 ± 0.02 to 0.86 ± 0.02%.[24]

It is important to increase the efficacy of tolvaptan in elderly and pediatric patients by enhancing its solubility and dissolution rate, and by improving patient compliance through reduction of side effects. Developing innovative dosage forms, such as ODTs rather than conventional solid dosage forms, increases patient compliance in older adults and pediatric patients. This study aimed to prepare CD-free and CD-containing SD formulations of tolvaptan using hydrophilic polymers and solubility enhancers by rotary evaporation, and to compare the solubility and dissolution rates of these formulations. After comparing the SD formulations for solubility and dissolution rate, the optimum formulation was prepared as an ODT using various excipients and rendered suitable for patient administration. To the best of our knowledge, no studies in the literature have reported formation of CD complexes with tolvaptan, preparation of SDs of these complexes, or development of ODT dosage forms.

MATERIALS AND METHODS

Materials

Tolvaptan was donated by Abdi İbrahim Pharmaceutical Company (Türkiye). Gelucire 44/14 was obtained from Gattefossé (France). HPβCD was provided by Roquette (France). βCD was purchased from Central Drug House (P) Ltd. (India). Polyvinylpyrrolidone (PVP) and Solutol HS-15 were acquired from BASF (Germany). SLS and methanol were purchased from Sigma-Aldrich (Germany).

Methods

Preparation of CD-free, βCD, and HPβCD-containing SD formulations

The phase solubility study was carried out in our previous work.[25] In the phase-solubility study of βCD and HPβCD, both CD molecules exhibited a linear increase in solubility with increasing CD concentration. Consequently, a 1:1 (drug:CD) inclusion complex was formed between the drug and CD, and an AL-type phase-solubility diagram was obtained. To produce CD-free SDs, 0.2 g of tolvaptan (2% *w/v*) was first dissolved in methanol; hydrophilic polymers (5–10% *w/v*) were then added, and the mixture was stirred with a magnetic stirrer for 1 h. Then, 0.5 g of solubility enhancers (5% *w/v*) were added and mixed until homogeneous. To produce SDs with CDs, 0.2 g of tolvaptan (2% *w/v*) was dissolved in methanol, and 0.2 g of CDs (2% *w/v*) were added and mixed using a magnetic stirrer for 24 h; hydrophilic polymers (5–10% *w/v*) were then added. Then, 0.5 g of solubility enhancers (5% *w/v*) were added and mixed until homogeneous (Table 1). The mixing continued for 1 h. A total of 10 mL of solution was obtained. The solvent from the final mixture was removed using a rotary evaporator (Büchi Rotavapor R-100) at 50 °C for 1 h. The resulting solid mass was milled into a powder.

Characterization of tolvaptan-CD complexes

After tolvaptan formed a complex with the CDs, X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analyses were performed to characterize the physical and chemical changes in the substances' structures.

Characterization of the SDs

Solubility studies

The solubilities of five tolvaptan-loaded SD formulations—CD-free (SD), βCD (βCD-SD), and HPβCD (HPβCD-SD)—were determined in distilled water at 37 °C. SD was placed in vials containing an excess of tolvaptan and was stirred in a water bath for 1 h. Tolvaptan solubility was determined spectrophotometrically at 260 nm (n = 3; Table 2).

Table 1. The composition of SD formulations containing CD-free, βCD, and HPβCD.

Formulation code	Tolvaptan (%)	PVP K90 (%)	PEO N80 (%)	Solutol HS-15 (%)	Gelucire 44/14 (%)	βCD (%)	HPβCD (%)
SD1	2	10	-	-	-	-	-
SD2	2	10	-	5	-	-	-
SD3	2	10	-	-	5	-	-
SD4	2	-	10	5	-	-	-
SD5	2	5	10	-	-	-	-
βCD-SD1	2	10	-	-	-	2	-
βCD-SD2	2	10	-	5	-	2	-
βCD-SD3	2	10	-	-	5	2	-
βCD-SD4	2	-	10	5	-	2	-
βCD-SD5	2	5	10	-	-	2	-
HPβCD-SD1	2	10	-	-	-	-	2
HPβCD-SD2	2	10	-	5	-	-	2
HPβCD-SD3	2	10	-	-	5	-	2
HPβCD-SD4	2	-	10	5	-	-	2
HPβCD-SD5	2	5	10	-	-	-	2

CD, cyclodextrins; HPβCD, 2-hydroxypropyl-beta-cyclodextrin; PVP, polyvinylpyrrolidone; SD, solid dispersion.

Table 2. Solubility results in distilled water of five SD formulations containing CD-free (SD), βCD (βCD-SD), and HPβCD (HPβCD-SD).

Solubility (mg/mL)	SD	βCD-SD	HPβCD-SD
Formulation code			
1	0.0293 ± 0.0102	0.0280 ± 0.0096	0.0277 ± 0.0105
2	0.1125 ± 0.0047	0.1114 ± 0.0147	0.2314 ± 0.0080
3	0.0668 ± 0.0019	0.0596 ± 0.0078	0.0313 ± 0.0080
4	0.0568 ± 0.0025	0.0080 ± 0.0017	0.0615 ± 0.0043
5	0.0339 ± 0.0058	0.0183 ± 0.0145	0.0374 ± 0.0058

CD, cyclodextrins; HPβCD, 2-hydroxypropyl-beta-cyclodextrin; PVP, polyvinylpyrrolidone; SD, solid dispersion.

XRD, DSC, and fourier transform infrared spectroscopy (FT-IR) analyses

XRD, DSC, and FT-IR analyses of the SDs were performed to examine changes in the physical and chemical structure of tolvaptan after solvent evaporation. The solid-state structural characteristics of tolvaptan, excipients, and SD were evaluated by XRD. An X-ray diffractometer (Rigaku ULTIMA IV) using Cu Kα radiation, operated at 30 mA and 40 kV, was used for XRD analysis. Scanning was performed at a scan rate of 1° min^{-1} over an angular range of 3–60° (2θ). The changes in the melting point of tolvaptan, active ingredient-polymer incompatibilities, and effects of amorphous-crystalline transformations on solubility were examined. DSC (Shimadzu, DSC-60, Japan) analysis was performed on pure tolvaptan, excipients, and SDs. In a nitrogen environment, the samples were heated from 25 °C to 360 °C at a rate of 10 °C/min. Tolvaptan, excipients, and SD were scanned in the range 650–4000 cm^{-1} using a spectrum 400 FT-IR spectrometer (Perkin Elmer, USA).

Scanning electron microscope (SEM) analyses

The surface morphologies of the SDs were examined using SEM. The SD formulations were coated with gold/palladium before SEM imaging. Images were taken from different parts of the SDs at 500x, 1000x, and 5000x magnification.

In vitro dissolution testing and release kinetics studies

After ODTs disintegrate in the mouth, the active ingredients dissolve in the gastrointestinal tract and are absorbed there, resulting in therapeutic efficacy in the organism. To estimate the dissolution rates of low-solubility active substances in the gastrointestinal system, *in vitro* dissolution tests were performed in buffers with pH 1.2, 4.5, and 6.8. The dissolution rate of tolvaptan in the SD formulation was measured in 900 mL of dissolution medium at 37 °C using the USP paddle method (50 rpm) specified by the FDA for tolvaptan. The dissolution rate studies were performed in distilled water, pH 1.2, 4.5, and 6.8 buffers, and SLS-containing buffers prepared by adding 0.22% SLS to these buffers. After the samples were filtered, their absorbance was measured using a validated ultraviolet (UV) spectrophotometric method, and the concentrations were calculated from the calibration equation.

Using DDSolver Software, the data from the dissolution rate study were fitted to the Weibull, Hixson-Crowell, Korsmeyer-Peppas, zero-order, first-order, and Higuchi models to identify potential mechanisms underlying the dissolution of tolvaptan from SDs. The adjusted coefficients of determination (r^2adj) obtained from the kinetic models were used to select the most suitable model (Table 3).[26]

Determination of drug content

Ten mg of the prepared SDs was precisely weighed and mixed until dissolved in 20 mL of distilled water (n = 3). After the SDs were dissolved, the solution was filtered through a membrane filter and tolvaptan was quantified by UV spectrophotometry at 260 nm.

Production of ODTs containing SD (SD-ODTs)

Various tablet formulations were prepared using the following excipients: Primogel, Ac-Di-Sol, Avicel-PH101, mannitol, xylitol, Ludiflash, Gantrez AN 119, Gantrez AN 139, Aerosil, Mg Stearate, sucrose, HPβCD, Na saccharin, Avicel PH102, Croscarmellose Na, Pharmaburst 500, sodium starch glycolate, Kollidon CL-SF (Tables 4 and 5). An amount of the SD formulation containing 15 mg of tolvaptan (equivalent to a commercial 15 mg tolvaptan tablet; 142.5 mg of SD formulation corresponds to 15 mg of tolvaptan) was weighed and then mixed with excipients. Then, the powder mass was compressed to produce SD-ODT formulations using an Erweka EK0. The optimum SD-ODT formulation was determined after examining the friability and disintegration time of the ODTs. Characterization studies of the tablets were performed.

Tablet characterization studies

Diameter-thickness-hardness in tablets

The diameters and thicknesses of 10 prepared tablets were measured using a digital micrometer (Mitutoyo), and the deviations were determined. The hardness values of 10 different tablets were measured using a tablet hardness tester. Determination of hardness is important in ODT pressing, since excessive tablet hardness may reduce porosity and prolong disintegration time.[27]

Table 3. The adjusted coefficient of determination (r^2adj) for kinetic models was obtained from experimental data in SLS-free and 0.22% SLS buffers of the HPβCD-SD2 formulation via DDsolver.

Kinetic models	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell	Weibull
Distilled water	0.602	0.998	0.945	0.977	0.995	0.999
pH 1.2	0.543	0.986	0.930	0.982	0.991	0.999
pH 4.5	0.907	0.999	0.995	0.993	0.992	1.000
pH 6.8	0.830	0.988	0.990	0.991	0.968	1.000
0.22% SLS-distilled water	0.496	0.962	0.932	0.998	0.932	0.985
0.22% SLS-pH 1.2	0.519	0.976	0.925	1.000	0.951	0.987
0.22% SLS-pH 4.5	0.511	0.927	0.872	0.998	0.835	0.984
0.22% SLS-pH 6.8	0.576	0.872	0.904	0.999	0.788	0.991

HPβCD, 2-hydroxypropyl-beta-cyclodextrin; SD, solid dispersion; SLS, sodium lauryl sulfate.

Table 4. Tablet formulations of solid dispersions prepared with super dispersing agents such as Primogel, Ac-Di-Sol, Avicel PH 101, Ludiflash.

mg / Number	SD	Primogel	Ac-Di-Sol	Avicel-PH 101	Mannitol	Xylitol	Ludiflash	Gantrez AN 119	Gantrez AN 139	Aerosil	Mg stearate	Total ODT weight
1	142.50	142.50	71.25	-	-	-	-	-	-	1.80	3.60	361.65
2	142.50	213.75	71.25	-	-	-	-	-	-	2.20	4.40	434.10
3	142.50	285	71.25	-	-	-	-	-	-	2.50	5	506.25
4	142.50	-	285	-	-	-	-	-	-	2.10	4.20	433.80
5	142.50	142.50	285	-	-	-	-	-	-	2.50	5	577.50
6	142.50	142.50	213.75	-	-	-	-	-	-	2.50	5	506.25
7	142.50	71.25	142.50	-	-	-	-	-	-	2.50	5	506.25
8	142.50	-	142.50	100	100	-		-	-	5	10	500
9	142.50	100	142.50	50	50	-	-	-	-	5	10	500
10	142.50	100	192.50	50	-	-	-	-	-	5	10	500
11	142.50	-	250	-	-	100	-	-	-	2.50	5	500
12	142.50	-	100	-	-	250	-	-	-	2.50	5	500
13	142.50	-	200	-	-	-	-	150	-	2.50	5	500
14	142.50	-	250	-	-	-	-	100	-	2.50	5	500
15	142.50	100	142.50	-	-	-	-	100	-	5	10	500
16	142.50	112.50	100	-	-	-	-	100	-	5	10	470
17	142.50	112.50	100		-	30	-	100	-	5	10	500
18	142.50	100	142.50	50		50	-	-	-	2.50	5	492.50
19	142.50	100	142.50	-	57.50	50	-	-	-	2.50	5	500
20	142.50	100	170	-	-	30	-	50	-	2.50	5	500
21	142.50	80	200	-	67.50	-	-	-	-	2.50	5	497.50
22	142.50	100	150	50	-	-	-	-	-	2.50	5	450
23	142.50	100	100	-	-	30	70	-	-	2.50	5	450
24	142.50	100	100	-	-	30	60	50	-	2.50	5	490
25	142.50	70	200	-	-	20	-	60	-	2.50	5	500
26	142.50	130	130	-	-	40	-	50	-	2.50	5	500
27	142.50	50	150	-	-	40	-	-	100	2.50	5	490
28	142.50	50	160	-	-	30	-	-	100	2.50	5	490
29	142.50	50	190	-	-	-	-	50	50	5	10	497.50
30	142.50	60	150	-	-	-	-	50	50	20	5	477.50
31	142.50	30	150	-	-	30	-	50	50	20	5	477.50
32	142.50	30	145	-	-	30	-	60	60	25	5	497.50
33	142.50	30	150	-	-	30	-	50	50	30	5	487.50
34	142.50	40	160	-	-	-	-	50	50	40	5	487.50
35	142.50	30	180	-	-	-	-	50	50	30	5	487.50
36	142.50	147.50	20	-	-	-	-	50	50	30	10	450
37	142.50	157.50	20	-	-	-	-	50	50	30	10	460
38	142.50	167.50	30	-	-	-	-	40	40	20	10	450

ODT, orally disintegrating tablets; SD, solid dispersion.

Table 5. Tablet formulations of solid dispersions prepared with super-dispersing agents such as Primogel, Ac-Di-Sol, Avicel PH 102, Croscarmellose Na, Pharmaburst 500, Na starch gluconate.

mg Number	SD	Primogel	Ac-Di-Sol	Mannitol	Ludiflash	Aerosil	Mg stearate	Sucrose	HPβCD	Na Saccharine	Avicel PH102	Croscarmellose Na	Pharmaburst 500	Sodium starch glycolate	Kollidon CL-SF	Total ODT weight
39	142.50	-	-	-	-	22.50	10	285	-	-	-	-	-	-	-	460
40	142.50	142.50	-	-	-	22.50	10	142.50	-	-	-	-	-	-	-	460
41	142.50	-	-	-	-	22.50	10	71.25	213.75	-	-	-	-	-	-	460
42	142.50	40	-	107.50	-	20	10	-	-	10	100	40	-	-	-	460
43	142.50	25	-	120	-	10	5	-	-	7.50	80	-	-	-	10	400
44	142.50	30	-	125	-	15	5	-	-	7.50	97.50	-	-	-	7.50	430
45	142.50	40	-	120	-	20	5	-	-	7.50	87.50	-	-	-	7.50	430
46	142.50	30	-	100	-	15	4	-	-	5	78.50	-	-	-	5	380
47	142.50	30	-	100	-	15	4	-	-	5	78.50	-	-	-	25	400
48	142.50	40	60	107.50	-		10	-	-	-	80	-	-	-	-	450
49	142.50	40	60	107.50	-	20	10	-	-	-	80	-	-	-	-	470
50	142.50	40	60	107.50	-	20	10	-	-	-	80	-	-	-	10	480
51	142.50	-	-	-	357.50	-	-	-	-	-	-	-	-	-	-	500
52	142.50	60	-	287.50	-	30	10	-	-	-	-	60	-	-	10	600
53	142.50	80	-	329.50	-	40	14	-	-	-	-	80	-	-	14	700
54	142.50	100	-	302.50	-	45	14	-	-	-	-	80	-	-	16	700
55	142.50	120	-	282.50	-	45	14	-	-	-	-	80	-	-	16	700
56	142.50	142.50	-	259	-	45	14	-	-	-	-	80	-	-	17	700
57	142.50	-	-	-	-	-	-	-	-	-	-	-	357.50	-	-	500
58	142.50	20	-	-	-	-	-	-	-	-	-	-	337.50	-	-	500
59	142.50	-	-	-	-	-	-	-	-	-	-	-	332.50	25	-	500

HPβCD, 2-hydroxypropyl-beta-cyclodextrin; SD, solid dispersion.

Friability in tablets

Friability was measured to assess the mechanical strength of the tablets. The European Pharmacopoeia Considers the friability of uncoated tablet formulations below 1% to be acceptable. Thus, the integrity of the tablet is maintained, and the mechanical strength is adequate.[28] The friability values of 20 tablets were determined after 100 rotations (4 minutes) using a friability tester (Pharma Test PTF 20E).

Disintegration test on tablets

Disintegration is an essential parameter of ODTs. According to the European Pharmacopoeia 9 monograph "Orodispersible tablets", ODTs must disperse in less than 3 min.[29] The test was performed in 900 mL of distilled water at 37 ± 0.5 °C.

Statistical analysis

All experimental measurements were performed in triplicate, and the data were expressed as the mean and standard deviation. Kinetic models were fitted to data from dissolution rate studies using the DDSolver Software (DDSolver: an add-in program for modeling and comparison of drug dissolution profiles).[30]

RESULTS

Characterization of tolvaptan-CD complexes

In the XRD analysis of pure βCD, sharp peaks corresponding to the crystal structure were observed at 4.53°, 9.09°, 12.57°, 17.91°, and 18.87° (Figure 1A). In the literature, XRD analyses often show a sharp peak characteristic of pure βCD.[31]

In the DSC study, the melting peaks of pure βCD, HPβCD, and tolvaptan at 300–350 °C disappeared as a result of the formation of the inclusion complex (Figure 1C and D). This indicates that tolvaptan and CDs form inclusion complexes.[32]

Characterization of the SDs

Solubility studies

SD formulations were prepared using the ingredients of five formulations coded HPβCD-NF1, HPβCD-NF2, HPβCD-NF3, HPβCD-NF4 and HPβCD-NF5, which were described in our previous study.[25] In the preformulation studies of SDs, polymer solutions containing CD (β-CD or HPβCD) and CD-free solutions were prepared. In these formulations, PVP K90 and PEO N80 were used as the hydrophilic polymers; Solutol HS-15 and Gelucire 44/14 as solubility enhancers; and βCD and HPβCD as complexing agents. A solubility study was performed on three SD formulations: CD-free (SD), βCD (βCD-SD), and HPβCD (HPβCD-SD). An increase in solubility relative to pure tolvaptan was expected owing to the use of a hydrophilic polymer, solubility enhancers, and the formation of an inclusion complex between tolvaptan and CD. In the solubility study, tolvaptan solubility in the HPβCD-SD2 formulation was highest. While the solubility of pure tolvaptan in distilled water was 0.0266 mg/mL, the solubility of tolvaptan in the HPβCD-SD2 formulation containing 2-HPβCD was 0.2314 mg/mL (Figure 2).

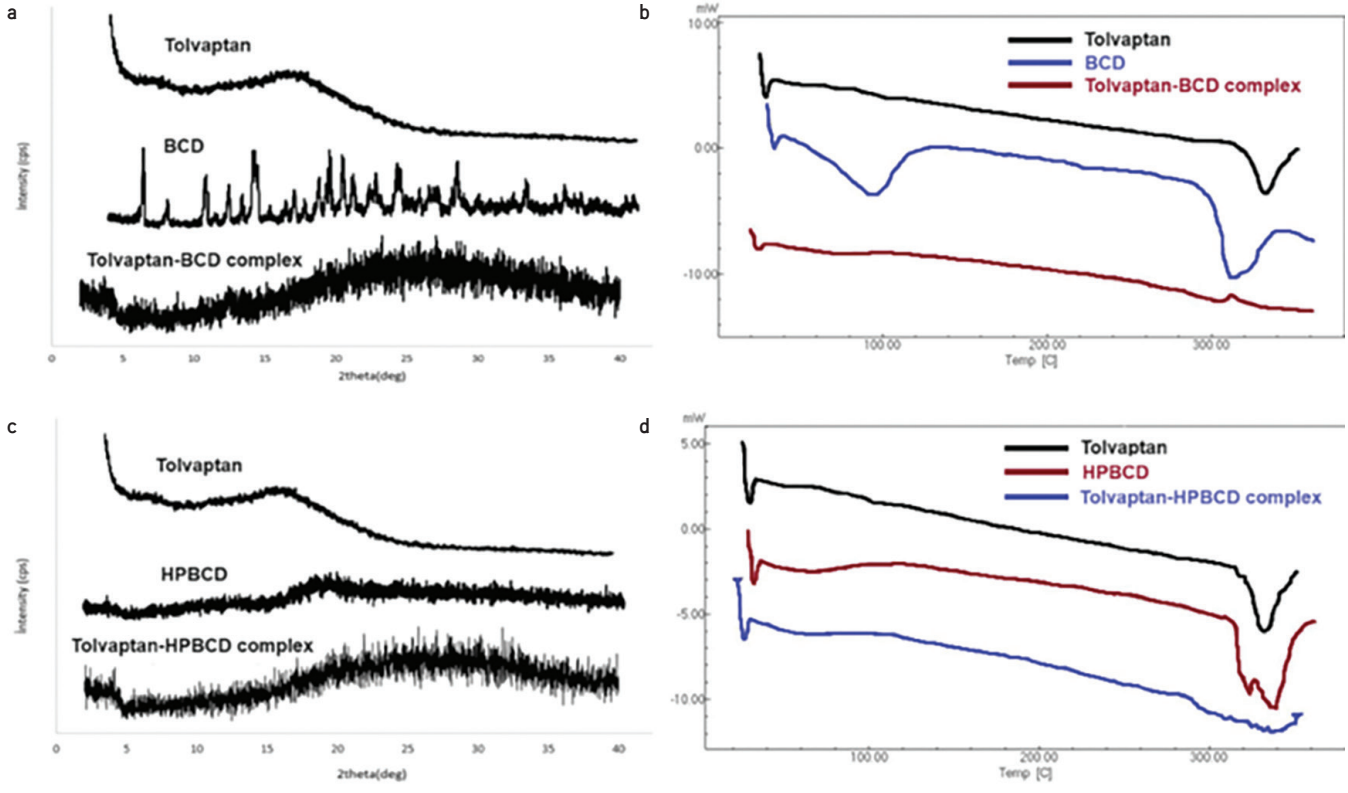


Figure 1. XRD diffractograms; (a) tolvaptan, βCD and tolvaptan-βCD complex, (b) tolvaptan, HPβCD and tolvaptan-HPβCD complex, and DSC thermograms; (c) tolvaptan, βCD and tolvaptan-βCD complex, (d) tolvaptan, HPβCD and tolvaptan-HPβCD complex.

HPβCD, 2-hydroxypropyl-beta-cyclodextrin; XRD, X-ray diffraction.

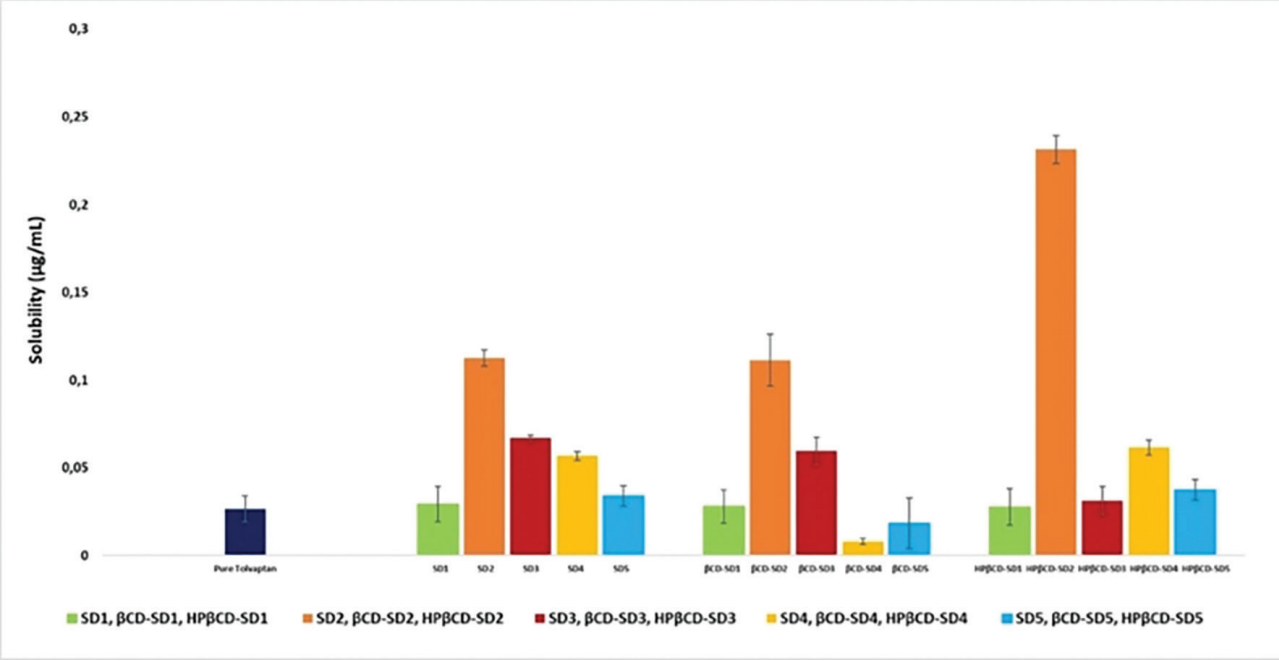


Figure 2. Solubility results in distilled water of pure tolvaptan and five SD formulations containing CD-free (SD), βCD (βCD-SD), and HPβCD (HPβCD-SD). CD, cyclodextrins; HPβCD, 2-hydroxypropyl-beta-cyclodextrin; SD, solid dispersion.

The solubility of tolvaptan in the HPβCD-SD2 formulation was approximately 8.7 times that of pure tolvaptan (Table 2). Therefore, HPβCD was chosen as the complexing agent, and HPβCD-SD2 was selected as the optimal SD formulation. Further studies have been conducted using this formulation.

XRD, DSC, and FT-IR analyses

XRD analyses of the five SD formulations were performed, and their diffractograms were obtained (Figure 3A). Tolvaptan did not produce any peaks due to the formation of an amorphous structure. No peaks were observed for the HPβCD-SD1 and HPβCD-SD2 formulations. The amorphous structure was preserved in these formulations. Characteristic sharp peaks of Gelucire 44/14 (19.219° and 23.360°) were observed in the HPβCD-SD3 formulation; characteristic sharp peaks of PEO N80 (19.140° and 23.340°) were observed in the HPβCD-SD4 formulation; and characteristic sharp peaks of PEO N80 were observed in the HPβCD-SD5 formulation.

DSC measurements were performed on the active ingredient HPβCD-SD2 and on the excipients of the HPβCD-SD2 formulation. The melting peaks of pure tolvaptan and HPβCD were observed between 300 and 350 °C. These peaks disappeared with the formation of the inclusion complexes. No peaks corresponding to tolvaptan or HPβCD were observed in the HPβCD-SD2 formulation (Figure 3B). FTIR analyses of tolvaptan, HPβCD-SD2, and the excipients were performed (Figure 3C). Pure PVP exhibited a broad absorption band between 3700 and 3000 cm^{-1} [33] and a C=O stretching band at 1655 cm^{-1}.[34] The C=O stretching vibration was observed at 1732 cm^{-1} in Solutol.[35] In pure HPβCD, a broad O-H stretching band was observed in the range of 3000–3600 cm^{-1}.[36] In the HPβCD-SD2 formulation, peaks corresponding to PVP were observed, whereas peaks corresponding to tolvaptan could not be distinguished because tolvaptan was present at a low concentration.

SEM analyses

SEM analyses of the SDs were performed. Solid masses without a specific shape were obtained for all the formulations. Studies have reported that SD masses are produced without a defined shape (Figure 4).[37-39]

In vitro dissolution test and release kinetics studies

Because of the low solubility of pure tolvaptan in SLS-free buffers, sink conditions could not be achieved. To provide sink conditions for pure tolvaptan in SLS-free buffers, 5.6–12.3 Liters of dissolution medium are needed. Sink conditions could not be achieved for pure tolvaptan in SLS-containing buffers (0.22% SLS at pH 1.2, 4.5, and 6.8). In the HPβCD-SD2 formulation, the solubilities of tolvaptan in SLS-containing and SLS-free buffers range from 0.2 to 1.6 mg/mL. In this case, studies were conducted under sink conditions in all buffers. The dissolution rate of the HPβCD-SD2 formulation was studied in buffers with and without 0.22% SLS. At least 90% of Tolvaptan in the formulation was dissolved in each buffer within 25 min (Figure 5).

The r^2adj values were compared as criteria for creating kinetic models from the data in the dissolution studies[40] and for determining the accuracy of these results. As r^2adj approaches 1, the kinetic model's similarity to the correct dissolution profile increases. After comparing these mathematical values, the dissolution profiles of the optimal formulation of HPβCD-SD2 in distilled water and in pH 1.2, 4.5, and 6.8 buffers were evaluated based on r^2adj, and the Weibull model was identified as the most

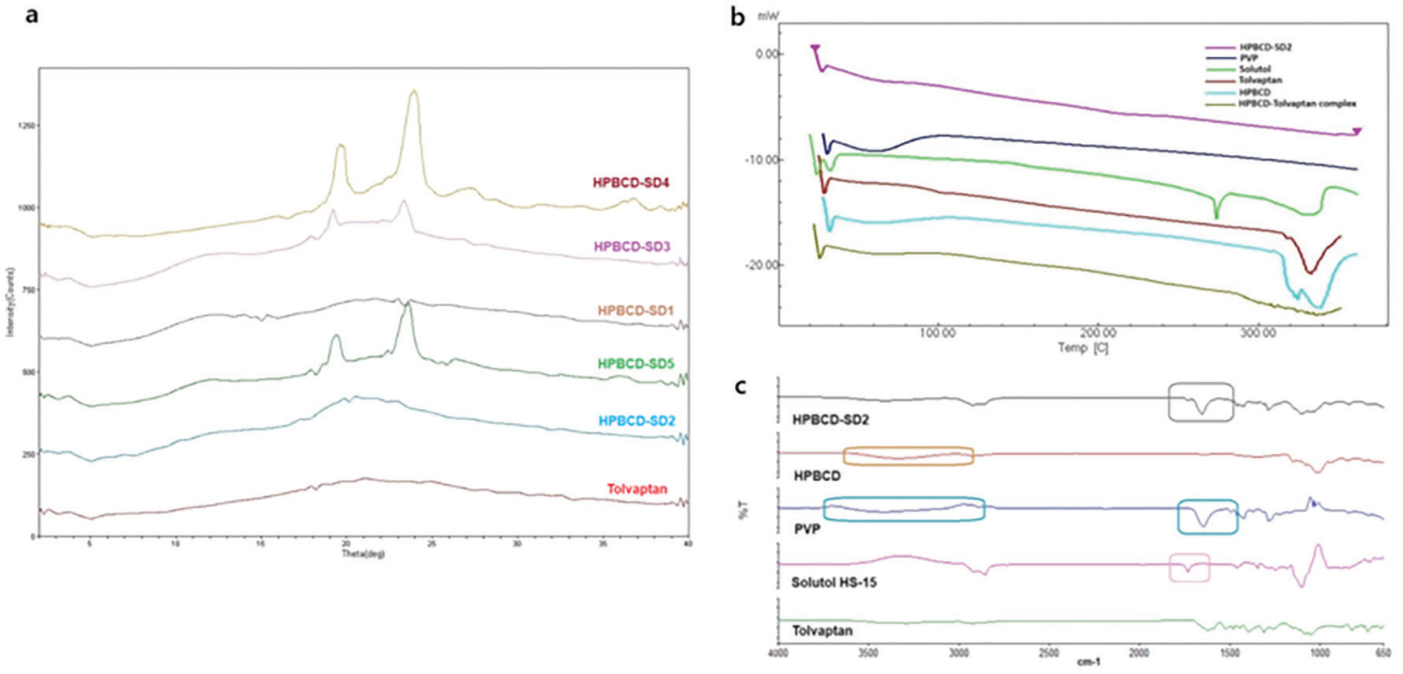


Figure 3. XRD diffractograms; (a) tolvaptan, HPβCD-SD1, HPβCD-SD2, HPβCD-SD3, HPβCD-SD4, HPβCD-SD5, and belonging to PVP, Solutol, tolvaptan, HPβCD, HPβCD-tolvaptan complex, and HPβCD-SD2; (b) DSC thermograms and (c) FTIR spectra.

DSC, differential scanning calorimetry; HPβCD, 2-hydroxypropyl-beta-cyclodextrin; PVP, polyvinylpyrrolidone; SD, solid dispersion; XRD, X-ray diffraction.

suitable kinetic model (Table 3). When the dissolution profiles of the HPβCD-SD2 formulation in buffers containing 0.22% SLS were evaluated based on r^2adj, the Korsmeyer–Peppas kinetic model was the most appropriate (Table 3).

In the Weibull model, β constants can provide information about the mechanisms underlying drug-release kinetics in SDs. It is predicted that when β < 0.75, Fickian diffusion is the dominant release mechanism; when 0.75 < ß < 1, Fickian diffusion and swelling are the dominant mechanisms; and when ß > 1, the release mechanism is complex.[41-43] The β values of the HPβCD-SD2 formulation in distilled water and in pH 1.2, pH 4.5, and pH 6.8 buffers were 0.7995, 0.6603, 0.7581, and 0.6975, respectively (Table 3).

Using the Korsmeyer-Peppas model, one can determine the type of diffusion by which the active substance is released from swelling and non-swelling polymeric drug carrier systems into the buffer. In the Korsmeyer-Peppas model, the exponent n was used to characterize the drug release mechanism. The release of the active substance is thought to occur via pseudo-Fickian diffusion when n < 0.5, via Fickian diffusion when n = 0.5, via anomalous diffusion when 0.5 < n < 1, and via non-Fickian diffusion when n = 1.[44,45] The n values in the 0.22% SLS buffers of the HPβCD-SD2 formulation were 0.3017, 0.2544, 0.2000, and 0.2330, respectively. Quantification was performed in the HPβCD-SD2 formulation and percent recovery was 97.95 ± 4.54%. This result indicates that almost all tolvaptan in the formulation was recovered, confirming successful loading.

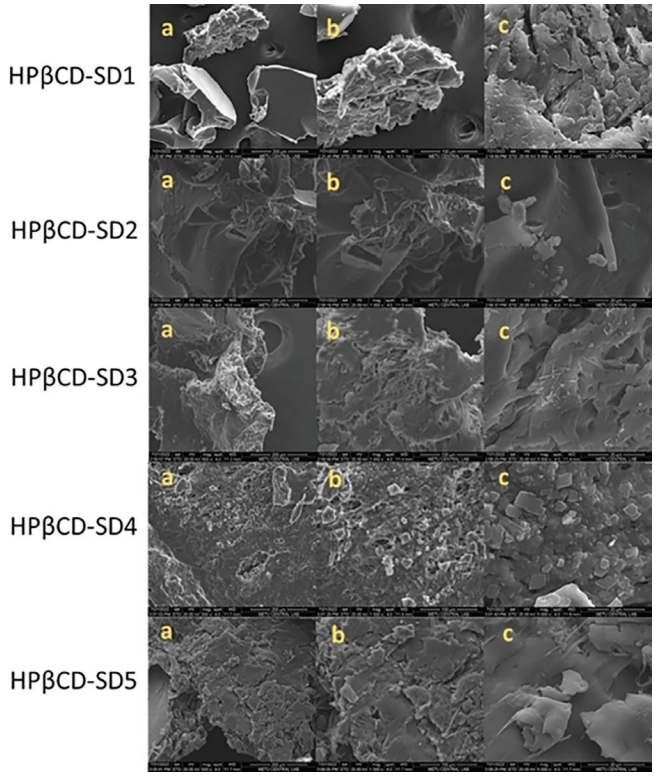


Figure 4. SEM images of HPβCD-SD1– HPβCD-SD5 [mag. (a) 500x, (b) 1000x, (c) 5000x].

HPβCD, 2-hydroxypropyl-beta-cyclodextrin; mag., magnification; SD, solid dispersion; SEM, scanning electron microscope.

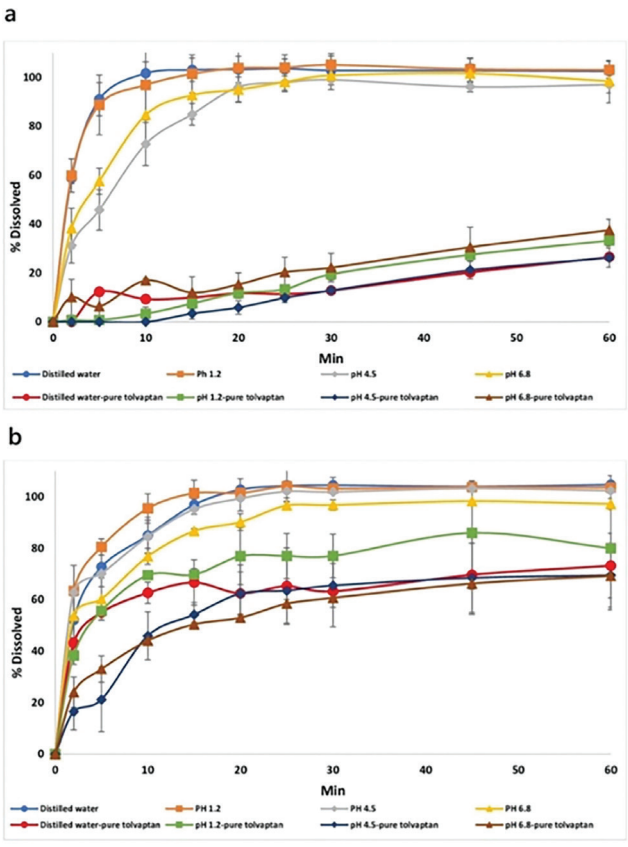


Figure 5. *In vitro* dissolution test of pure tolvaptan and HPβCD-SD2 formulation in (a) SLS-free buffers, (b) 0.22% SLS buffers.

HPβCD, 2-hydroxypropyl-beta-cyclodextrin; SD, solid dispersion; SLS, sodium lauryl sulfate.

Production of ODTs

The ODTs were pressed after adding superdispersing agents to the SDs (Tables 4 and 5). Based on comparisons of friability and disintegration time among the prepared ODTs, formulation 59, which showed ≤1% friability and a disintegration time ≤180 s, was selected as the optimal formulation (SD-ODT; Table 6).

Characterization studies of ODTs

The mean weight, diameter, and thickness of the prepared SD-ODT were 497.50 ± 0.53 mg, 3.99 ± 0.01 mm, and 12.13 ± 0.01 mm, respectively. The standard deviations of these parameters were small, indicating that the values were generally similar. The hardness of SD-ODT was 33.66 ± 2.94 n and did not prolong the disintegration time; SD-ODT dispersed within 94.00 ± 8.19. The friability was 0.88%.

DISCUSSION

This study aimed to increase the efficacy of tolvaptan by enhancing its water solubility and dissolution rate and improving patient compliance. For this purpose, SD formulations were prepared and ODTs were developed from them. During this process, characterization studies were performed, and the results were evaluated. The solvent evaporation method was preferred for SD preparation because it is a fast, inexpensive, and simple method for obtaining the product.

According to the XRD results, βCD was found in crystalline form.[46] Tolvaptan did not exhibit sharp peaks and was present in an amorphous form. The tolvaptan–βCD complex did not exhibit sharp peaks; the sharp peaks of βCD disappeared upon complex formation. In this case, the tolvaptan–βCD complex exhibited an amorphous structure. In the XRD analysis of pure 2-HPβCD, no sharp peaks corresponding to the crystal structure were observed (Figure 1A). Reported XRD analyses of pure HPβCD do not exhibit sharp peaks.[47] Similarly, the tolvaptan-HPβCD complex did not exhibit sharp peaks; instead, a peak pattern indicating formation of an amorphous complex was observed (Figure 1B).

The solubility study showed that preparing the HPβCD-SD2 formulation increased the solubility of tolvaptan compared with pure tolvaptan (Figure 2). Solubility results indicated that tolvaptan in the SDs of complexes prepared with HPβCD had higher solubility than in complexes prepared with CD-free βCD. The increase in the solubility of tolvaptan is thought to be due to the use of a hydrophilic polymer such as PVP K90, the use of a solubility enhancer such as Solutol HS-15, and the formation of an inclusion complex between tolvaptan and CD. Hydrophilic polymers, such as PVP K90, encapsulate active substances between polymer chains, thereby imparting hydrophilicity to the encapsulated substances.[48] Solutol HS-15 is a non-ionic surfactant.[49,50] Surfactants form micelles around active substances, thereby increasing their solubility. Active substances form complexes with the internal cavities of 2-HPβCD, increasing their solubility in water owing to the hydrophilic outer surface of 2-HPβCD. Additionally, the presence of surfactants, such as SLS, in the solubility medium increased dissolution. The solubility of tolvaptan in the HPβCD-SD2 formulation was higher in SLS-containing distilled water and pH 1.2 buffers than in SLS-containing pH 4.5 and 6.8 buffers. At pH 4.5 and 6.8, the sodium ions from disodium hydrogen phosphate and SLS produce a common-ion effect. Because of the common-ion effect, the solubility of active substances is lower than expected.[51] It is thought that the lower solubility of tolvaptan in the HPβCD-SD2 formulation in SLS-containing media at pH 4.5 and 6.8, compared with its solubility in SLS-containing distilled water and in pH 1.2 environments, is due to the common-ion effect. In a study, SD formulations of silnidipine were prepared. Solubility studies in SD formulations were carried out in distilled water and in a pH 6.8 medium containing 0.2% SLS. In SD containing a 1:3 silnidipine:PVP ratio, the solubilities of pure silnidipine were 0.6371 µg/mL in distilled water and 1.0051 µg/mL in a pH 6.8 medium containing 0.2% SLS. The solubility of silnidipine increased when SLS was added to the medium.[52]

DSC analysis showed no observable tolvaptan or HPβCD peaks in the HPβCD-SD2 formulation (Figure 3B). This may be due to the formation of an inclusion complex between tolvaptan and the CDs. Previous studies have reported that the DSC melting peak of the active substance disappears after the formation of the inclusion complex.[12,53]

Table 6. Friability and disintegration time results of tablets prepared with SD.

Number	Friability (%)	Disintegration time (s)	ODT weight (mg)	Number	Friability (%)	Disintegration time (s)	ODT weight (mg)
1	>1	>180	361.65	31	>1	>180	477.50
2	>1	>180	434.10	32	>1	>180	497.50
3	>1	>180	506.25	33	>1	145	487.50
4	>1	>180	433.80	34	>1	>180	487.50
5	>1	>180	577.50	35	>1	>180	487.50
6	>1	>180	506.25	36	>1	150	450
7	>1	>180	506.25	37	>1	>180	460
8	>1	>180	500	38	>1	>180	450
9	>1	>180	500	39	>1	>180	460
10	>1	>180	500	40	>1	>180	460
11	>1	>180	500	41	>1	>180	460
12	>1	>180	500	42	>1	90	460
13	>1	>60	500	43	>1	>180	400
14	>1	>60	500	44	>1	180	430
15	>1	>60	500	45	>1	>180	430
16	>1	150	470	46	>1	130	380
17	>1	>180	500	47	0.91	>180	400
18	>1	>180	492.50	48	>1	>180	450
19	>1	>180	500	49	>1	120	470
20	>1	>180	500	50	>1	>180	480
21	>1	180	497.50	51	>1	>180	500
22	>1	>180	450	52	>1	>180	600
23	>1	>180	450	53	>1	90	700
24	>1	>180	490	54	>1	175	700
25	>1	>180	500	55	>1	155	700
26	>1	>180	500	56	>1	165	700
27	>1	>180	490	57	>1	>180	500
28	>1	>180	490	58	0.43	>180	500
29	>1	>180	497.50	**59**	**0.88**	**94**	**500**
30	>1	>180	477.50				

ODT, orally disintegrating tablets; SD, standard deviation.

In the dissolution rate study of pure tolvaptan in buffer media tested (with or without 0.22% SLS), except in 0.22% SLS in distilled water, sink conditions could not be achieved in these media because of the low solubility of pure tolvaptan. Because sink conditions were not maintained, the dissolution rate was slow, and the percentage of tolvaptan dissolved did not reach 100% after 60 min. In 0.22% SLS in distilled water, tolvaptan dissolved rapidly during the second minute, reaching 43.19% dissolution, but the dissolution rate decreased thereafter. This is thought to be caused by the rapid dissolution and transformation of amorphous tolvaptan into a crystalline form. The dissolution rate study of the HPβCD-SD2 formulation was conducted in buffers with and without 0.22% SLS. At least 90% of tolvaptan in the formulation dissolved within 25 min in all buffers, both with and without 0.22% SLS (Figure 5). Rapid dissolution depends on the formulation containing a hydrophilic polymer with a large surface area, a solubility enhancer, and an inclusion complex with CDs.[54,55] Tolvaptan

was dispersed among the hydrophilic PVP K90 polymer chains, resulting in an increase in its hydrophilicity. Solutol HS-15 formed micelles with tolvaptan. The outer surfaces of the micelles formed by surrounding tolvaptan molecules are hydrophilic. In addition, tolvaptan formed an inclusion complex with 2-HPβCD, which exhibited hydrophilic properties. In our study, tolvaptan demonstrated hydrophilicity in three respects. Because tolvaptan is hydrophilic, it rapidly dissolves upon contact with the hydrophilic dissolution medium. In this case, dissolution occurred rapidly. The faster dissolution rate in buffers containing 0.22% SLS compared with buffers without SLS is thought to result from surfactants such as SLS reducing the surface tension between liquid and solid surfaces, thereby increasing the interaction between lipophilic drugs and the hydrophilic solvent medium.[56] The presence of surfactants, such as Solutol HS-15 in the formulation and SLS in the dissolution medium, accelerated the dissolution of tolvaptan from the HPβCD-SD2 formulation.

A study observed that SDs of indomethacin prepared with water-soluble carriers, such as PEG 4000 or Gelucire 50/13, alter its crystallinity depending on the carrier type and polymer amount. It has also been reported to contribute to an increase in the release rate of the drug, a decrease in the size and agglomeration of the particles, an increase in their wettability, and a decrease in the crystallinity of the drug.[57] In another study aimed at increasing the solubility and dissolution rate of itraconazole, a drug with low water solubility, researchers used hydrophilic polymers such as polyvinyl acetal diethylaminoacetate (AEA®) and Eudragit® E 100 with the SD method. The dissolution rate of itraconazole from the tablets obtained using SDs prepared by spray drying increased, with more than 90% of the drug released within 5 min. SDs prepared with AEA® or Eudragit® E 100 at a 1:1 (*w/w*) drug/hydrophilic polymer ratio showed approximately a 70-fold increase in dissolution rate, compared to the commercial product.[58]

The HPβCD-SD2 formulation released the drug into the buffer at pH 1.2 and 6.8 via Fickian diffusion. In contrast, drug release in distilled water and in pH 4.5 buffer proceeded via Fickian diffusion and swelling mechanisms (Table 3). Zhu et al.[59] prepared SDs of resveratrol, a compound with poor water solubility using hydrophilic polymers (Eudragit RS and polyethylene glycol 600). In the release study, the r^2adj values of the kinetic models were examined; the highest r^2adj was observed for the Weibull kinetic equation. Weibull kinetics is a model that describes the dissolution behavior of matrix systems.[60] They stated that the Weibull model is an empirical model used in rapid-release and SR drug-release systems, and that formulation[59] exhibits burst release of resveratrol. Tolvaptan in the HPβCD-SD2 formulation was released into buffers containing 0.22% SLS by pseudo-Fickian diffusion. In a study, SDs of gliclazide were prepared using PVP K90. By fitting the *in vitro* dissolution data to various release kinetic models, correlation coefficient (r) values were obtained, and it was determined that the Korsmeyer-Peppas kinetic model was the most suitable. The n-value was 0.7249, indicating that the release occurred via non-Fickian diffusion.[61] Tolvaptan was dissolved from the HPβCD-SD2 formulation by non-swelling-controlled matrix diffusion.

Formulations coded 13, 14, 15, 16, 33, 36, 42, 46, 49, 53, 54, 55, 56, and 59 met the European Pharmacopoeia Requirement for a disintegration time of <3 min. Formulations coded as 47, 58, and 59 had acceptable friability (<1%). In the preformulation study of ODTs, the following superdisintegrants were used (Table 5): Primogel, 30 mg in formulation coded 47; Pharmaburst 500, 357.50 mg in formulation coded 57; a mixture of Primogel (20 mg) and Pharmaburst 500 (337.50 mg) in formulation coded 58; and a mixture of sodium starch glycolate (25 mg) and Pharmaburst 500 (332.50 mg) in formulation coded 59. The additional use of sodium starch glycolate as a superdisintegrants in the 59-coded formulation, unlike in the 57-coded formulation, had a positive effect on disintegration time and friability. Although using Primogel as a superdisintegrants in formulation codes 47 and 58 improved friability, it did not improve disintegration time. After evaluating dispersion time and friability of the formulations, formulation code 59 was selected as the final ODT formulation (Table 6). The disintegration time of ODT formulations generally increases with hardness. As the compression force increased, powder particles slid past one another and formed a tablet with a denser structure, making it more difficult for water to penetrate the tablet and disperse within the medium. To ensure that the disintegration time was shorter by 3 min, a lower compressive force was applied. However, a low compression force resulted in loose structures and increased tablet friability (>1%). Preparing the optimal ODT that met the appropriate dispersion time, friability, and hardness criteria was challenging. To overcome this challenge, SD can be developed using the lower-molecular-weight polymer PVP K30 instead of PVP K90. In a study, tablet formulations were prepared using ibuprofen-containing pellets. Tablets were pressed by applying forces of 5, 10, and 15 kN to the mixtures, and the effects of these forces on the disintegration time and friability were examined. With increasing tablet compression force, disintegration time increased, whereas friability decreased. consequently, it was reported that, as the compression force increased, a more compact mass was formed.[62] In a study, SDs were prepared with meloxicam, PVP K30, crospovidone, and SLS, and ODT formulations were developed from the resulting solid mass. The optimized ODT showed a hardness of 48 ± 4.3 N. This formulation achieved rapid disintegration in 19 ± 2 s.[63] If the high-molecular-weight PVP K90 is used in SD-ODT, it may cause longer disintegration times than observed in this study. Moreover, this value is below 3 min, meeting the requirement for ODTs. in addition, the friability value was 0.88%, which is less than 1% and is in accordance with the pharmacopoeial standard.

CONCLUSION

In this study, SDs were prepared by the solvent evaporation method to improve the solubility and dissolution rate of tolvaptan, an active substance classified as BCS Class IV. solubility and dissolution rates of the resulting SD formulations were evaluated. After detailed characterization of these

formulations, several ODT formulations were developed from the selected optimum formulation (HPβCD-SD2) using different superdisintegrants. The characterization studies demonstrated that SD-ODT meets pharmacopeial standards for ODTs. For geriatric and pediatric patients who have difficulty swallowing or refuse to do so, a tolvaptan-containing dosage form has been developed that can be easily administered without water and has a faster onset of action. Further *in vivo* studies are needed to demonstrate the efficacy of the final formulation.

Ethics

Ethics Committee Approval: There is not required for ethical approval.

Informed Consent: There is not required for informed consent.

Footnotes

Authorship Contributions

Concept: A.A.K., S.T., F.A., Design: A.A.K., S.T., F.A., Data Collection or Processing: A.A.K., S.T., F.A., Analysis or Interpretation: A.A.K., S.T., F.A., Literature Search: A.A.K., S.T., F.A., Writing: A.A.K., S.T., F.A.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Nikghalb LA, Singh G, Singh G, Kahkeshan KF. Solid dispersion: methods and polymers to increase the solubility of poorly soluble drugs. J Appl Pharm Sci. 2012;2:170-175.
2. Chiou WL, Riegelman S. Preparation and dissolution characteristics of several fast-release solid dispersions of griseofulvin. J Pharm Sci. 1969;58:1505-1510.
3. Gurunath S, Pradeep Kumar S, Basavaraj NK, Patil PA. Amorphous solid dispersion method for improving oral bioavailability of poorly water-soluble drugs. J Pharm Res. 2013;6:476-480.
4. Chiou WL, Riegelman S. Pharmaceutical applications of solid dispersion systems. J Pharm Sci. 1971;60:1281-1302.
5. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today. 2007;12:1068-1075.
6. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012;2012:195727.
7. Vo CL, Park C, Lee BJ. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. Eur J Pharm Biopharm. 2013;85:799-813.
8. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev. 2007;59:645-666.
9. Lu X, Zhang J, Zuo W, Cheng B, Dong R, Wang W, Zhang L, Li M. A dissolving microneedle patch loaded with plumbagin/hydroxypropyl-beta-cyclodextrin inclusion complex for infected wound healing. Colloids Surf B Biointerfaces. 2025;246:114377-114377.
10. Jug M, Mura PA. Grinding as solvent-free green chemistry approach for cyclodextrin inclusion complex preparation in the solid state. Pharmaceutics. 2018;10:4-4.
11. Manne ASN, Hegde AR, Raut SY, Rao RR, Kulkarni VI, Mutalik S. Hot liquid extrusion assisted drug-cyclodextrin complexation: a novel continuous manufacturing method for solubility and bioavailability enhancement of drugs. Drug Deliv Transl Res. 2021;11:1273-1287.
12. Ozdemir N, Erkin J. Enhancement of dissolution rate and bioavailability of sulfamethoxazole by complexation with beta-cyclodextrin. Drug Dev Ind Pharm. 2012;38:331-340.
13. Chinwala M. Recent formulation advances and therapeutic usefulness of orally disintegrating tablets (ODTs). Pharmacy (Basel). 2020;8:4-4.
14. Slavkova M, Breitkreutz J. Orodispersible drug formulations for children and elderly. Eur J Pharm Sci. 2015;75:2-9.
15. Hirani JJ, Rathod DA, Vadalia KR. Orally disintegrating tablets: a review. Trop J Pharm Res. 2009;8:2-2.
16. Bahrainian S, Abbaspour M, Kouchak M, Taghavi Moghadam P. A review on fast dissolving systems: from tablets to nanofibers. Jundishapur J Nat Pharm Prod. 2016;12:262-267.
17. Rahman Z, Zidan AS, Khan MA. Risperidone solid dispersion for orally disintegrating tablet: its formulation design and non-destructive methods of evaluation. Int J Pharm. 2010;400:49-58.
18. Kugler JP, Hustead T. Hyponatremia and hypernatremia in the elderly. Am Fam Physician. 2000;61:3623-3630.
19. Buffington MA, Abreo K. Hyponatremia: a review. J Intensive Care Med. 2016;31:223-236.
20. Zmily HD, Daifallah S, Ghali JK. Tolvaptan, hyponatremia, and heart failure. Int J Nephrol Renovasc Dis. 2011;4:57-71.
21. Miyazaki T, Fujiki H, Yamamura Y, Nakamura S, Mori T. Tolvaptan, an orally active vasopressin V2-receptor antagonist - pharmacology and clinical trials. Cardiovasc Drug Rev. 2007;25:1-13.
22. Lee JH, Lee GW. Formulation approaches for improving the dissolution behavior and bioavailability of tolvaptan using SMEDDS. Pharmaceutics. 2022;14:2-2.
23. Ramesh K, Shekar BC, Khadgapathi P, Bhikshapathi DVRN. Design and evaluation of tolvaptan solid dispersions using hot-melt extrusion and spray drying technique–a comparative study. Der Pharmacia Lettre. 2015;7:218-231.
24. Prasad SG, Gupta VR, Yasasvi Narayan G, Vijaya K, Tamilselvan A, Siva Subramanian N. Formulation and evaluation of fast dissolving tablet of tolvaptan. J Glob Trends Pharm Sci. 2015;6:2403-2410.
25. Kara A, Tort S, Yücel Ç, Acartürk F. *In vitro* evaluation and characterization of tolvaptan/cyclodextrin complex loaded electrospun nanofibers. Braz J Pharm Sci. 2025;61:e24314-e24314.
26. Abdul Rasool BK, Mohammed AA, Salem YY. The optimization of a dimenhydrinate transdermal patch formulation based on the quantitative analysis of *in vitro* release data by DDSolver through skin penetration studies. Sci Pharm. 2021;89:3-3.
27. Mostafa HF, Ibrahim MA, Sakr A. Development and optimization of dextromethorphan hydrobromide oral disintegrating tablets: effect of formulation and process variables. Pharm Dev Technol. 2013;18:454-463.
28. Debunne A, Vervaet C, Mangelings D, Remon JP. Compaction of enteric-coated pellets: influence of formulation and process parameters on tablet properties and *in vivo* evaluation. Eur J Pharm Sci. 2004;22:305-314.
29. Ghourichay MP, Kiaie SH, Nokhodchi A, Javadzadeh Y. Formulation and quality control of orally disintegrating tablets (ODTs): recent advances and perspectives. Biomed Res Int. 2021;2021:6618934-6618934.

30. Tunçel E, Tort S, Han S, Yücel Ç, Tırnaksız F. Development and optimization of hydrogel-forming microneedles fabricated with 3D-printed molds for enhanced dermal diclofenac sodium delivery: a comprehensive *in vitro*, ex vivo, and *in vivo* study. Drug Deliv Transl Res. 2025;15:2116-2145.

31. Bhattacharjee T, Rahman S, Deka D, Purkait MK, Chowdhury D, Majumdar G. Synthesis and characterization of exfoliated beta-cyclodextrin functionalized graphene oxide for adsorptive removal of atenolol. Mater Chem Phys. 2022;288:1-8.

32. Williams RO 3rd, Mahaguna V, Sriwongjanya M. Characterization of an inclusion complex of cholesterol and hydroxypropyl-beta-cyclodextrin. Eur J Pharm Biopharm. 1998;46:355-360.

33. Tantishaiyakul V, Kaewnopparat N, Ingkatawornwong S. Properties of solid dispersions of piroxicam in polyvinylpyrrolidone. Int J Pharm. 1999;181:143-151.

34. Adeli E. Irbesartan-loaded electrospun nanofibers-based PVP K90 for the drug dissolution improvement: fabrication, *in vitro* performance assessment, and *in vivo* evaluation. J Appl Polym Sci. 2015;132:27-27.

35. Varshosaz J, Ghassami E. Enhancement of dissolution rate of fenofibrate by spray drying technique: comparison of Eudragit E100, Solutol HS15 and hydroxypropyl cellulose as carriers. Farmacia. 2015;63:433-445.

36. Mihajlovic T, Kachrimanis K, Graovac A, Djuric Z, Ibric S. Improvement of aripiprazole solubility by complexation with 2-hydroxypropyl-beta-cyclodextrin using spray drying technique. AAPS PharmSciTech. 2012;13:623-631.

37. Dong L, Mai Y, Liu Q, Zhang W, Yang J. Mechanism and improved dissolution of glycyrrhetinic acid solid dispersion by alkalizers. Pharmaceutics. 2020;12:1-1.

38. Febriyenti F, Rahmi S, Halim A. Study of gliclazide solid dispersion systems using PVP K-30 and PEG 6000 by solvent method. J Pharm Bioallied Sci. 2019;11:262-267.

39. Yadav PS, Kumar V, Singh UP, Bhat HR, Mazumder B. Physicochemical characterization and *in vitro* dissolution studies of solid dispersions of ketoprofen with PVP K30 and dmannitol. Saudi Pharm J. 2013;21:77-84.

40. Skiba M, Skiba M, Milon N, Bounoure F, Fessi H. Preparation and characterization of amorphous solid dispersions of nimesulide in cyclodextrin copolymers. J Nanosci Nanotechnol. 2014;14:2772-2779.

41. Mircioiu C, Voicu V, Anuta V, Tudose A, Celia C, Paolino D, Fresta M, Sandulovici R, Mircioiu I. Mathematical modeling of release kinetics from supramolecular drug delivery systems. Pharmaceutics. 2019;11:3-3.

42. Adibkia K, Selselehjonban S, Emami S, OsouliBostanabad K, BarzegarJalali M. Electrosprayed polymeric nanobeads and nanofibers of modafinil: preparation, characterization, and drug release studies. Bioimpacts. 2019;9:179-188.

43. Tuğcu-Demiröz F, Saar S, Kara AA, Yıldız A, Tunçel E, Acartürk F. Development and characterization of chitosan nanoparticles loaded nanofiber hybrid system for vaginal controlled release of benzydamine. Eur J Pharm Sci. 2021;161:105801-105801.

44. Adibkia K, Ghajar S, OsouliBostanabad K, Balaei N, Emami S, BarzegarJalali M. Novel gliclazide electrosprayed nanosolid dispersions: physicochemical characterization and dissolution evaluation. Adv Pharm Bull. 2019;9:231-240.

45. Rezaei A, Nasirpour A, Tavanai H, Fathi M. A study on the release kinetics and mechanisms of vanillin incorporated in almond gum/polyvinyl alcohol composite nanofibers in different aqueous food simulants and simulated saliva. Flavour Fragr J. 2016;31:442-447.

46. Abarca RL, Rodriguez FJ, Guarda A, Galotto MJ, Bruna JE. Characterization of betacyclodextrin inclusion complexes containing an essential oil component. Food Chem. 2016;196:968-975.

47. Kim JS. Study of flavonoid/hydroxypropylbetacyclodextrin inclusion complexes by UVVis, FTIR, DSC, and Xray diffraction analysis. Prev Nutr Food Sci. 2020;25:449-456.

48. Hrib J, Sirc J, Hobzova R, Hampejsova Z, Bosakova Z, Munzarova M, Michalek J. Nanofibers for drug delivery – incorporation and release of model molecules, influence of molecular weight and polymer structure. Beilstein J Nanotechnol. 2015;6:1939-1948.

49. Bergonzi MC, Vasarri M, Marroncini G, Barletta E, Degl'Innocenti D. Thymoquinone-Loaded Soluplus®-Solutol® HS15 Mixed Micelles: preparation, *in vitro* characterization, and effect on the SHSY5Y cell migration. Molecules. 2020;25:20-20.

50. da Fonseca Antunes AB, De Geest BG, Vervaet C, Remon JP. Gelucire 44/14 based immediate release formulations for poorly watersoluble drugs. Drug Dev Ind Pharm. 2013;39:791-798.

51. Serajuddin AT, Sheen PC, Augustine MA. Common ion effect on solubility and dissolution rate of the sodium salt of an organic acid. J Pharm Pharmacol. 1987;39:587-591.

52. Mohana M, Vijayalakshmi S. Development and characterization of solid dispersionbased orodispersible tablets of cilnidipine. BeniSuef Univ J Basic Appl Sci. 2022;11:1-1.

53. Ficarra R, Ficarra P, Di Bella MR, Raneri D, Tommasini S, Calabrò ML, Gamberini MC, Rustichelli C. Study of betablockers/betacyclodextrins inclusion complex by NMR, DSC, Xray and SEM investigation. J Pharm Biomed Anal. 2000;23:33-40.

54. Kalia A, Poddar M. Solid dispersions: an approach towards enhancing dissolution rate. Int J Pharm Pharm Sci. 2011;3:9-19.

55. Ruan LP, Yu BY, Fu GM, Zhu DN. Improving the solubility of ampelopsin by solid dispersions and inclusion complexes. J Pharm Biomed Anal. 2005;38:457-464.

56. Vemula VR, Lagishetty V, Lingala S. Solubility enhancement techniques. Int J Pharm Sci Rev Res. 2010;5:41-51.

57. ElBadry M, Fetih G, Fathy M. Improvement of solubility and dissolution rate of indomethacin by solid dispersions in Gelucire 50/13 and PEG4000. Saudi Pharm J. 2009;17:217-225.

58. Jung JY, Yoo SD, Lee SH, Kim KH, Yoon DS, Lee KH. Enhanced solubility and dissolution rate of itraconazole by a solid dispersion technique. Int J Pharm. 1999;187:209-218.

59. Zhu W, Fan W, Zhang X, Gao M. Sustainedrelease solid dispersion of highmeltingpoint and insoluble resveratrol prepared through hot melt extrusion to improve its solubility and bioavailability. Molecules. 2021;26:16-16.

60. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67:217-223.

61. Biswal S, Sahoo J, Murthy PN. Physicochemical properties of solid dispersions of gliclazide in polyvinylpyrrolidone K90. AAPS PharmSciTech. 2009;10:329-334.

62. Abbaspour MR, Sadeghi F, Afrasiabi Garekani H. Design and study of ibuprofen disintegrating sustained-release tablets comprising coated pellets. Eur J Pharm Biopharm. 2008;68:747-759.

63. Dehghani H, Taheri A, Homayouni A. Design, optimization and evaluation of orally disintegrating tablet of meloxicam using its menthol based solid dispersions. Curr Drug Deliv. 2017;14:709-717.

Turk J Pharm Sci 2026;23(1):22-30
DOI: 10.4274/tjps.galenos.2026.86132

ORIGINAL ARTICLE

Assessment of the Potential Health Risks Associated with the Toxic and Essential Elements Content in Tea Samples Marketed in Türkiye

Muhammed HAMİTOĞLU[1]*, Mert ŞAHİN[1], Gülşah ESEN[1], Sinem HELVACIOĞLU[2], Ahmet AYDIN[1]

[1]Yeditepe University Faculty of Pharmacy, Department of Toxicology, İstanbul, Türkiye
[2]Vrije Universiteit Brussel Faculty of Medicine and Pharmacy, Department of Pharmaceutical and Pharmacological Sciences, Brussels, Belgium

ABSTRACT

Objectives: Tea is a widely consumed beverage that may contain both essential and toxic metals, raising nutritional and health concerns. This study evaluated the transfer of selected essential and toxic metals from black tea leaves to their infusions and assessed the associated health risks and nutritional contributions.

Materials and Methods: Black tea leaves and their infusions from eight commercially available brands sold in Türkiye were analyzed. Toxic elements [arsenic (As), aluminium, cadmium (Cd), lead (Pb)] were quantified using inductively coupled plasma mass spectrometry, while essential elements [copper, iron (Fe), magnesium (Mg), zinc] were quantified using flame atomic absorption spectrometry. Health risks were evaluated using the Estimated Daily Intake, the Target Hazard Quotient (THQ), the Hazard Index (HI), and the Incremental Lifetime Cancer Risk (ILCR).

Results: In dry tea leaves, aluminum was the predominant toxic element (12.6–25.3 mg/g), followed by Pb (21.0–39.0 µg/g); As and Cd were present at lower levels. Mg (523.0–1421.0 µg/g) and Fe (263.1–445.5 µg/g) were the most abundant essential elements. Metal concentrations in tea infusions were significantly lower than those in dry leaves ($p < 0.05$), indicating limited transfer during brewing. In infusions, aluminum (0.5–1.0 mg/g) and Mg (88.5–413.4 µg/g) were the dominant toxic and essential elements, respectively. All THQ values and the combined HI were below 1, indicating no significant non-carcinogenic risk. ILCR values for Pb were below 10^{-6}, whereas those for As ranged from 10^{-6} to 10^{-4}, indicating a moderate carcinogenic risk for As.

Conclusion: The transfer of metals from tea leaves to infusions is limited, likely due to chelation and complexation during brewing. Under typical consumption conditions, black tea infusions were within established safety limits, with risk indices below thresholds of concern. While As warrants continued monitoring, essential elements contribute only a small fraction of the recommended daily intakes. The small sample size is a limitation.

Keywords: Cancer slope factor, estimated Incremental Lifetime Cancer Risk, Hazard Index, Hazard Quotient, metal contamination

INTRODUCTION

Tea, derived from the leaves of *Camellia sinensis* L., is among the most widely consumed non-alcoholic beverages worldwide. Its popularity is attributed to its therapeutic benefits, invigorating qualities and mild stimulant effects.[1] Tea consumption has been associated with reduced serum cholesterol levels, inhibition of low-density lipoprotein oxidation and a lower risk of cardiovascular diseases and cancer.[2] Tea leaves and processed tea products possess a complex chemical composition, including tannins, flavonols, alkaloids, proteins, amino acids, enzymes, aroma compounds, vitamins, minerals and essential elements.[3] Tea plants are known to accumulate various metals from the soil, and numerous studies have shown that cultivation conditions can influence metal uptake in tea leaves. Metals

***Correspondence:** mohammad.saz@yeditepe.edu.tr, **ORCID-ID:** orcid.org/0000-0002-4545-0756
Received: 24.12.2024, **Accepted:** 20.02.2026 **Epub:** 10.04.2026 **Publication Date:** 13.07.2026

Cite this article as: Hamitoğlu M, Şahin M, Esen G, Helvacıoğlu S, Aydın A. Assessment of the potential health risks associated with the toxic and essential elements content in tea samples marketed in Türkiye. Turk J Pharm Sci. 2026;23(1):22-30

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

such as copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg) are essential for biological functions and play critical roles in enzymatic activity, oxygen transport, and cellular metabolism. However, their health effects may vary depending on intake levels, as both deficiencies and excessive exposure can lead to adverse outcomes.[1]

In contrast, arsenic (As), aluminium (Al), cadmium (Cd), and lead (Pb) are classified as non-essential elements due to their inherent toxicity, even at low concentrations. Toxic effects can also occur when the level of an essential element becomes excessively elevated, leading to harmful impacts on the central nervous system, blood constituents, and vital organs such as the lungs, liver, and kidneys, which can contribute to various disease conditions, including mental disorders.[4] Given the importance of both essential and toxic elements in tea, numerous studies have investigated their concentrations in tea leaves and infusions.[5-7] However, the metal content of tea may vary considerably due to environmental factors, geographic characteristics, agricultural practices, and processing conditions. Therefore, monitoring metal levels in tea products remains essential from both nutritional and toxicological perspectives.

While determining elemental concentrations and comparing them with regulatory limits is important, such an approach alone may not fully capture potential public health implications. A comprehensive human health risk assessment provides a more informative framework by integrating exposure levels with toxicological reference values. In this context, Türkiye represents a particularly relevant case, as it is among the leading countries worldwide in per capita tea consumption, with black tea constituting a major component of the daily diet across all age groups. Tea cultivation in Türkiye, particularly in the Black Sea region, is carried out under distinct regional and environmental conditions. Despite widespread consumption and potential dietary exposure to metals, data integrating both elemental composition and health risk assessment for commercially available black tea products in Türkiye remain limited.

Therefore, this study aims to assess the levels of essential and toxic elements in eight commercially available bagged black tea samples and their infusions sold in Türkiye and to evaluate the associated human health risks. Non-carcinogenic and carcinogenic risks related to toxic element exposure were assessed using the Target Hazard Quotient (THQ), Hazard Index (HI), and Incremental Lifetime Cancer Risk (ILCR). For essential elements, estimated dietary intakes were compared with established toxicological reference values, including the Tolerable Daily Intake (TDI), Provisional TDI (PMTDI), reference dose (RfD), and Tolerable Upper Intake Level (UL).

MATERIALS AND METHODS

Sample collection and preparation

The content of heavy metals and essential elements in black teas available in Turkish markets that were widely accepted and commonly consumed was analyzed in this study. A total of eight commercially available bagged black tea brands were selected for analysis. To achieve this, black tea bags with similar production dates were sourced from the same market. The samples were stored at room temperature, kept away from direct sunlight, and were confirmed to be undamaged prior to analysis.

Analytical procedures

For metal determination, 0.1 g of each dry tea sample was placed in a Teflon vessel and digested in a microwave-assisted acid digestion system (Anton Paar, Germany). The digestion process utilized 4.5 mL of 20% nitric acid (Merck, Germany) and 0.5 mL of hydrogen peroxide (Sigma, Germany).

For tea infusions, three black tea bags (weighing approximately 6 g in total) from each sample were steeped in 200 mL of distilled water at room temperature for 5 minutes according to brewing guidelines recommended by the tea industry. This standardized brewing duration was selected to ensure comparability between samples and to represent realistic exposure conditions for routine tea consumption.[8] During this time, the mixture was stirred at 400 rpm using a magnetic stirrer. After brewing, the tea bags were held above the beaker for 1 minute to allow excess liquid to drain. The resulting infusion was capped and stored in a refrigerator until analysis. No additional preparation was required for the infusion samples prior to measurement.

Elemental analysis was performed using a validated, in-house accredited method. Toxic elements (As, Al, Cd, Pb) were quantified using Inductively Coupled Plasma Mass Spectrometry (Thermo Scientific ICAP Q Series, Germany),[9] while essential elements (Cu, Fe, Mg, Zn) were determined using Flame Atomic Absorption Spectrometry (Analytik Jena, Germany).[10]

Risk assessment for consumers

The Estimated Daily Intake (EDI) of toxic elements from tea infusion samples was determined using the equation:

EDI = [concentration × ingestion rate (IR) × exposure frequency (EF) × exposure duration (ED)]/[body weight (BW) × averaging time]

Where EDI is the EDI (mg/kg/day), C is the metal concentration in tea infusion samples and IR is the daily consumption of tea per capita in Türkiye (10 g/day). EF is the EF (365 day/year), ED is the ED for adults (54 years), BW is the average BW of consumers (60 kg) and AT indicates the average lifetime (70 years × 365 days).[9,11] In the risk assessment, per-capita daily tea consumption was assumed to be 10 g/day (dry weight), a conservative estimate for a high-tea-consuming population such as Türkiye. Türkiye has one of the highest per capita tea consumption rates in the world, with annual average consumption exceeding 3.2 kg per person, corresponding to an average of several cups of tea per day.[12]

To assess potential non-carcinogenic health risks, the THQ was calculated for each toxic element (THQ = EDI/RfD). RfD is the oral RfD for the intended trace element (As = 0.0003, Al = 1, Pb = 0.002 mg/kg/day).[13] If the THQ value is less than one, it

indicates no significant risk of non-carcinogenic adverse health effects to consumers. Additionally, to evaluate the potential cumulative hazard resulting from expose to multiple metal, HI was calculated ($HI = THQ_{As} + THQ_{Pb} + THQ_{Cd} + THQ_{Al}$).

Cancer risk was estimated as the lifetime probability of developing cancer due to exposure to potential carcinogens. The ILCR was calculated as the product of the EDI and the cancer slope factor (CSF), the latter being derived from the dose–response curve for ingestion of the toxicant, according to the formula:

$ILCR = EDI \times CSF$[14]

For essential elements, the risk assessment compared the EDI of Zn, Fe, Cu, and Mg from black tea consumption with established toxicological safety guidelines, including the TDI, PMTDI, RfD, and UL.

Statistical analysis

All analyses were conducted using SPSS software version 21.0. The concentrations of toxic and essential elements were expressed as mean ± standard deviation. One-way analysis of variance, followed by Tukey's post-hoc HSD test, was used to determine significant differences between the mean concentrations in dry tea leaves and tea infusions. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Metal concentrations in tea leaves and tea infusions

In this study, eight different black tea bag samples, representing the most frequently consumed brands in Türkiye, were selected for analysis. The toxic and essential element contents per tea bag sample are presented in Table 1.

The levels of both toxic and essential elements were roughly similar among the tested brands. The most abundant toxic metals in tea leaves were Al (12.6–25.3 mg/g) and Pb (21.0–39.0 µg/g), whereas the most prevalent essential elements were Mg (523.0–1421.0 µg/g) and Fe (263.1–445.5 µg/g). The contents of Cu, Zn, As, and Cd were in the ranges of 25.0–29.3 µg/g, 37.2–51.3 µg/g, 0.7–1.6 µg/g, and 0.6–2.1 µg/g, respectively.

Toxic and essential elements were also measured in tea infusions prepared following the recommended steeping time of 5 minutes for black tea. The concentrations in infusion samples for each brand are shown in Table 2; Figure 1 compares the average element concentrations in dry tea leaves and in the corresponding infusions.

As shown in Table 2, the primary toxic metal found in the infusion samples was Al, with concentrations ranging from 0.5 to 1.0 mg/g. On the other hand, the predominant essential element was Mg, with concentrations ranging from 88.5 to 413.4 µg/g. The total contents of other elements determined in tea infusions are presented in the following order: Fe (39.4 to 70.0 µg/g), Cu (13.5 to 17.8 µg/g), Zn (9.4 to 16.9 µg/g), As (0.1 to 0.3 µg/g), and Pb (0.1 to 0.5 µg/g). The concentrations of Cd in the infusion samples were below the limit of detection of the available analytical technique (3.7 ppb).

All elements showed a significant decrease ($p < 0.05$) in infusions compared with those in dry leaves. The extraction efficiency of each element was calculated as the concentration of the element in tea infusions divided by its total concentration in black tea leaves (Figure 1).

Health risk assessment

Table 3 presents the EDI, THQ, and HI values calculated for the non-carcinogenic risk assessment of tea infusions. The EDI was the highest for Al among all brands under investigation, while it was relatively similar for As and Pb. The lowest THQ was observed for Pb, while As and Al exhibited similar THQ values, both higher than that for Pb. None of the individual THQs for Al, As, and Cd exceed the value of 1 in any of the tea infusion samples examined. This observation implies that the consumption of the analyzed tea infusions does not pose a significant risk of non-carcinogenic health effects. The HI, which accounts for the combined impact of consuming multiple toxic elements, also remains below 1 (Table 3). The obtained values indicate that Al contributes the most to the

Table 1. Toxic and essential element level in tea leaves in tea bag samples (mean + standard deviation).

	Toxic elements level				Essential elements level			
Sample	As (µg/g)	Al (mg/g)	Cd (µg/g)	Pb (µg/g)	Zn (µg/g)	Fe (µg/g)	Cu (µg/g)	Mg (µg/g)
Brand A	1.0 ± 0.1	18.1 ± 0.9	1.0 ± 0.1	21.0 ± 1.1	37.2 ± 1.4	263.1 ± 5.0	25.0 ± 0.7	523.0 ± 35.4
Brand B	1.0 ± 0.1	17.5 ± 0.9	0.9 ± 0.0	21.0 ± 1.1	42.6 ± 0.8	350.4 ± 14.0	28.5 ± 0.8	1134.2 ± 53.0
Brand C	1.6 ± 0.0	25.2 ± 1.3	1.0 ± 0.1	26.0 ± 1.3	48.6 ± 0.8	445.5 ± 5.1	28.7 ± 1.6	1421.0 ± 33.5
Brand D	1.6 ± 0.1	25.3 ± 1.3	1.2 ± 0.1	28.0 ± 1.4	47.7 ± 6.0	416.7 ± 7.0	27.8 ± 1.7	952.0 ± 30.3
Brand E	1.5 ± 0.1	23.3 ± 1.2	2.1 ± 0.1	25.0 ± 1.3	51.3 ± 6.1	417.6 ± 20.7	27.7 ± 0.9	1049.3 ± 49.5
Brand F	0.7 ± 0.0	12.6 ± 0.6	0.9 ± 0.0	21.0 ± 1.1	48.9 ± 6.4	360.9 ± 9.6	29.3 ± 0.6	1161.0 ± 24.3
Brand G	0.7 ± 0.1	13.1 ± 0.7	0.6 ± 0.0	21.0 ± 1.1	44.4 ± 1.4	330.3 ± 12.8	29.1 ± 0.9	1263.2 ± 33.0
Brand H	1.3 ± 0.1	27.0 ± 1.3	1.3 ± 0.1	39.0 ± 2.0	40.8 ± 5.4	400.5 ± 9.9	27.7 ± 1.0	937.0 ± 36.5

Al, aluminium; As, arsenic; Cd, cadmium; Cu, copper; Fe, iron; Mg, magnesium; Pb, lead; Zn, zinc.

Table 2. Toxic and essential elements level in tea infusion samples (mean + standard deviation).

Sample	Toxic elements level				Essential elements level			
	As (µg/g)	Al (mg/g)	Cd (µg/g)	Pb (µg/g)	Zn (µg/g)	Fe (µg/g)	Cu (µg/g)	Mg (µg/g)
Brand A	0.3 ± 0.01	0.9 ± 0.04	<LOD	0.1 ± 0.01	9.4 ± 0.35	39.4 ± 2.20	17.2 ± 0.49	209.8 ± 13.61
Brand B	0.3 ± 0.03	0.7 ± 0.04	<LOD	0.3±0.02	11.4 ± 0.14	52.3 ± 0.13	17.7 ± 0.50	292.0 ± 19.26
Brand C	0.2 ± 0.01	0.8 ± 0.04	<LOD	0.1±0.01	12.8 ± 0.71	55.8 ± 1.17	16.5 ± 0.49	124.0 ± 15.76
Brand D	0.2 ± 0.01	0.8 ± 0.04	<LOD	0.2±0.01	14.2 ± 1.06	65.4 ± 0.10	17.5 ± 0.45	88.5 ± 13.78
Brand E	0.2 ± 0.01	0.8 ± 0.04	<LOD	0.4±0.02	13.6 ± 1.32	70.0 ± 1.47	17.8 ± 0.90	136.5 ± 18.02
Brand F	0.1 ± 0.01	0.5 ± 0.03	<LOD	0.5±0.02	14.4 ± 1.12	61.3 ± 0.49	15.0 ± 0.69	280.4 ± 11.89
Brand G	0.1 ± 0.01	0.7 ± 0.04	<LOD	0.5 ± 0.02	16.9 ± 1.02	67.2 ± 1.79	13.5 ± 0.46	413.4 ± 7.86
Brand H	0.1 ± 0.01	1.0 ± 0.05	<LOD	0.2 ± 0.01	13.1 ± 1.06	67.7 ± 5.09	13.7 ± 0.32	92.1 ± 7.10

Al, aluminium; As, arsenic; Cd, cadmium; Cu, copper; Fe, iron; LOD, limit of detection; Mg, magnesium; Pb, lead; Zn, zinc.

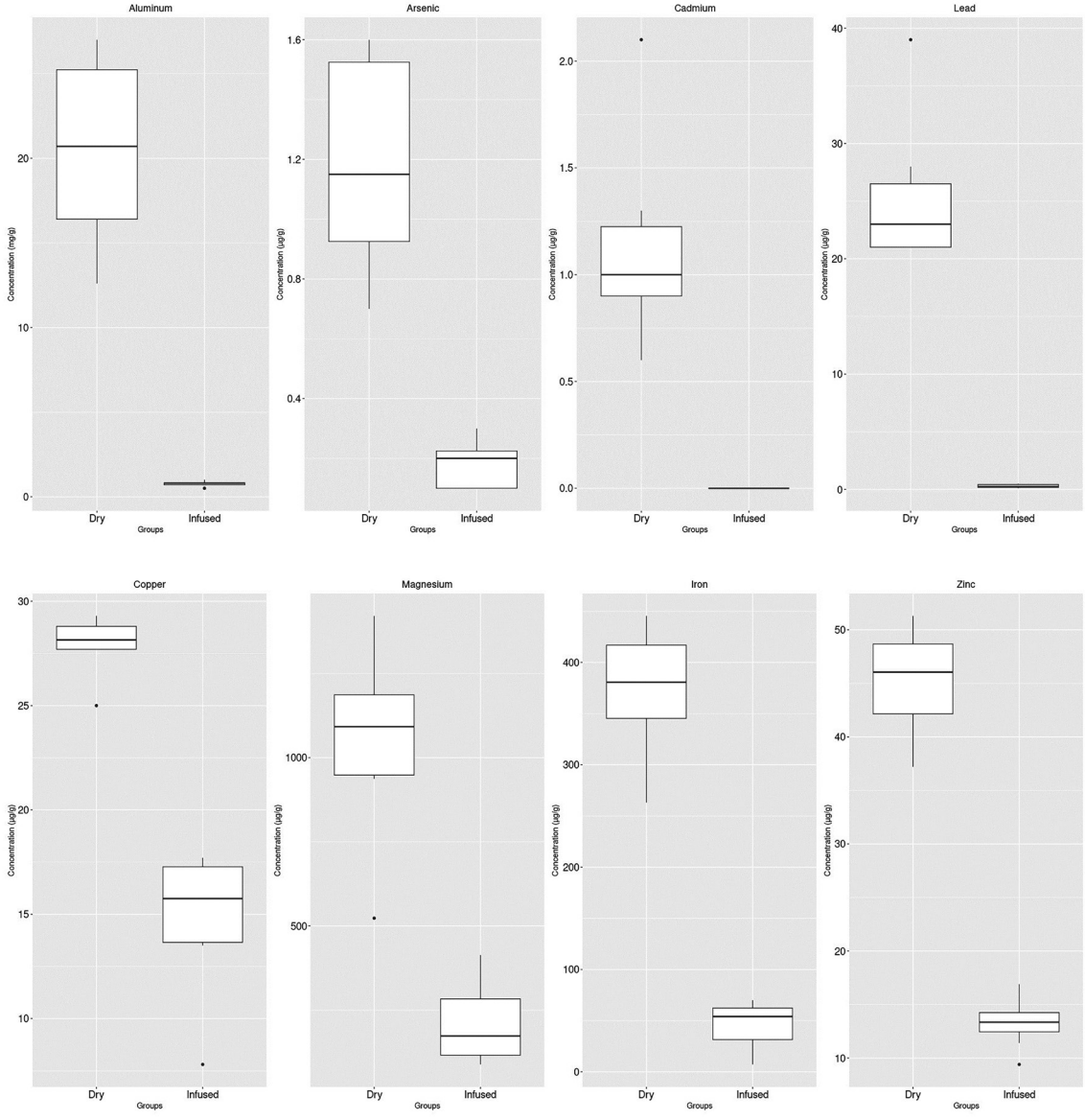


Figure 1. The comparison of toxic and essential elements content in dry leaves and infusions of black tea.

HI of the examined tea infusion samples, at 50%. As contributes 41% and Pb contributes 9% (Figure 2). The carcinogenic risk of As and Pb via the consumption of tea infusions was evaluated and is summarized in Table 4. The assessment was based on the CSF values for inorganic As (1.5 per mg/kg/day) and Pb (0.0085 mg/kg/day). An ILCR above 10^{-4} indicates high risk, while values below 10^{-6} indicate minimal risk; values between 10^{-6} and 10^{-4} indicate moderate risk. In this study, ILCR values for Pb were below 10^{-6} in all tea infusion samples, indicating negligible carcinogenic risk. ILCR values for As ranged from 10^{-6} to 10^{-4}, indicating a moderate risk.

For the essential elements, the EDI of Fe, Cu, Zn, and Mg from tea infusions was compared with their toxicological reference values (PMTDI, TDI, UL, or RfD) as presented in Table 5. The results indicated that the average EDI for these elements constituted a very small fraction (0.01%–5%) of the established reference values for a 60-kg person. Therefore, the intake of these essential elements through tea infusions is unlikely to pose any significant health risk to the average consumer.

DISCUSSION

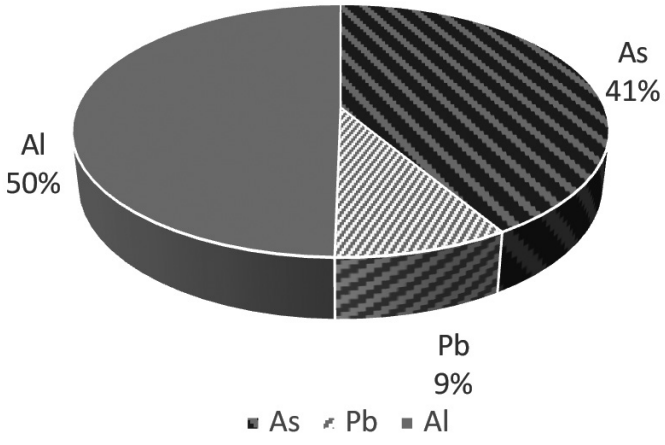


Figure 2. Average percentage contribution of Al, As and Pb to the HI.
Al, aluminium; As, arsenic; HI, Hazard Index; Pb, lead.

Previous studies have reported varying concentrations of toxic and essential elements in black tea from different regions. Matsuura et al.[6] reported an average Al level of 0.8 mg/g in black tea bags commercially available in Japan. Tang et al.[15] determined Al concentration of 0.48 mg/g in dry tea leaves marketed in Hangzhou, China. In another study, the ranges of Al and Pb in mature tea leaves from Puan County, China were found to be 4.3–10.4 mg/g and 0.56–1.26 µg/g, respectively.[16] Ghale-Askari et al.[17] reported the mean concentrations of 1.96, 0.048, 2.195 and 0.03 µg/g for Pb, As, Al, and Cd in the tea leave samples marketed in Neishabour city, Iran. In another study the Pb, Cd, As and Cu concentrations in the Sri Lankan and Indian black tea were 0.14, 0.017, 0.057, 11.29 mg/kg, and 0.21, 0.02, 0.067, 14.56 mg/kg, respectively.[18] The quality of tea brands available in the retail market in Saudi Arabia were evaluated based on their metal contents in the study conducted by Al-Oud.[19] The concentrations of the toxic elements Pb and Cd in tea leaves were 0.03–14.84 µg/g and below the detectable limit of 0.37 µg/g, respectively. Among essential elements, Fe was the most abundant (326–1155 µg/g), followed by Zn (26.7–53.9 µg/g) and Cu (22.1–40.7 µg/g). More recently, Rahmani et al.[20] reported that imported black teas in Mashhad, Iran contained concentrations ranging from 6.81 to 35.21 µg/g for Cu, 14.61 to 164.84 µg/g for Zn, 0.038 to 1.62 µg/g for Pb and 0.006 to 0.19 µg/g for Cd.

Overall, our findings for the toxic element content of dry tea leaves were higher than those reported in previous studies. The highest level of Al among previous studies was found in the samples obtained from factories located in Giresun, Trabzon and Rize in Türkiye.[21] The average Al content of the tea was found to be between 8.9 and 14.1 mg/g in Rize, between 8.2 and 11.2 mg/g in Trabzon, and between 8.8 and 15.7 mg/g in Giresun.

The elevated concentrations of toxic elements in tea samples from Türkiye are likely attributable to specific cultivation conditions and environmental factors, including soil characteristics, climate, altitude, and rainfall. Previous studies have indicated that significant quantities of metals may translocate from the soil to tea leaves.[22] In particular, tea

Table 3. EDI, THQ and HI values of the studied toxic elements in tea infusions.

Sample	EDI/THQ				
	As	Al	Cd*	Pb	HI
Brand A	3.7E-05/0.12	1.2E-01/0.12	NA	1.7E-05/0.01	0.25
Brand B	3.5E-05/0.12	9.02E-02/0.09	NA	4.3E-05/0.02	0.23
Brand C	3.0E-05/0.10	1.1E-01/0.11	NA	1.7E-05/0.01	0.21
Brand D	2.4E-05/0.08	1.0E-01/0.10	NA	2.1E-05/0.01	0.19
Brand E	2.2E-05/0.07	9.8E-02/0.10	NA	4.3E-05/0.02	0.19
Brand F	2.1E-05/0.07	7.4E-02/0.087	NA	6.4-05/0.03	0.17
Brand G	1.9E-05/0.06	9.6E-02/0.10	NA	6.0E-05/0.03	0.19
Brand H	1.7E-05/0.06	1.3E-01/0.13	NA	2.6E-05/0.01	0.20

*The risk assessment for Cd was not conducted because its concentration was found to be below the limit of detection in all investigated infusion samples. Al, aluminium; As, arsenic; Cd, cadmium; EDI, Estimated Daily Intake; HI, Hazard Index; NA, not applicable; THQ, Target Hazard Quotient.

leaves grown in acidic soils tend to accumulate higher levels of metals, a phenomenon linked to the soil's acidic properties.[23] Özyazıcı et al.[24] further corroborated this by reporting the acidic pH range (3.14–6.39) of soils in regions of Türkiye where tea cultivation is prevalent.

It is crucial to emphasize that the retention of elements within an organism depends on two factors: the overall element content within tea leaves and the fraction that is extracted into the infusion.[25] Therefore, in this study, levels of both toxic and essential elements in tea infusions were analyzed following the recommended 5-minute steeping time for black tea. It has been acknowledged that the quantity of elements extracted into the tea infusions primarily depends on whether the compound is tightly bound to the matrix or more soluble in the employed solution.[26] Hence, the amount of minerals in tea infusions is determined by the extraction efficiencies and the total concentration of metals within the tea leaves.[25]

According to their extraction efficiencies, elements are classified into highly (>55%), moderately (22–55%) and poorly extractable elements (<20%).[27] In this study, after 5 minutes of brewing, the derived extraction efficiencies for the elements under investigation indicated that As, Al, Cd, Pb, Fe, and Mg were poorly extractable. Zn is in the moderately extractable range, whereas Cu is in the highly extractable range. Similarly, in a study conducted by Salahinejad and Aflaki[25] on black tea samples from the Iranian market, Cd, Pb and Fe were categorized as poorly extractable, while Zn fell into the moderately extractable category. In contrast to our results, Al and Mg were classified as moderately extractable, and Cu was categorized as poorly extractable in that study. The discrepancy in findings between the two studies may be attributable to the longer brewing period (30 minutes) used in the other study, compared with the shorter period (5 minutes) used in our investigation. In the present study, Al exhibited the highest EDI among the investigated elements, while As and Pb showed comparable EDI values. Conversely, Pb had the lowest THQ, whereas Al and As displayed similar and relatively higher THQ values. In order to calculate the EDI and THQ, an established oral RfD is essential. The RfD is an estimate of the daily intake of a substance, considered safe for the general population, including sensitive groups, over a lifetime without causing adverse health effects. The RfD is derived from studies identifying the No Observed Adverse Effect Level, the Lowest Observed Adverse Effect Level, or the benchmark dose, with safety factors applied to address uncertainties in the available data.[28]

Al has an oral RfD of 1 mg/kg/day. Dietary sources account for over 90% of non-occupational human exposure to aluminum.[13] Experimental animal studies have reported potential neurotoxic effects associated with prolonged exposure to Al. Growing scientific evidence suggests a potential connection between aluminum's neurotoxic effects and the onset of Alzheimer's disease. Although this association remains primarily correlational, aluminum has also been implicated in age-related cognitive decline and in neurodegenerative disorders such as Parkinson's disease. Research in humans has predominantly focused on dialysis patients, especially those suffering from dialysis encephalopathy, to explore the impact of aluminum exposure on brain function.[29]

The established oral RfD for inorganic As is 0.0003 mg/kg/day. Similar to Al, the primary source of As for individuals not exposed through occupational settings is their diet. Seafood commonly carries elevated levels of As, primarily in the form of the less harmful organic As species.[13] Aside from food, beverages such as tea may be significant sources of dietary exposure to toxic elements in daily life. The Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO) has established a provisional tolerable weekly intake (PTWI)

Table 4. The ILCR for As and Pb was analysed in tea infusions.

Sample	ILCR*	
	As	Pb
Brand A	5.5E-05	1.5E-07
Brand B	5.3E-05	3.6E-07
Brand C	4.4E-05	1.5E-07
Brand D	3.6E-05	1.8E-07
Brand E	3.2E-05	3.6E-07
Brand F	3.1E-05	5.5E-07
Brand G	2.9E-05	5.1E-07

*ILCR was calculated by multiplying the EDI by the CSF. Oral CSF for inorganic As and Pb is 1.5 and 8.5 x 10^{-3} mg/kg/day, respectively.[26] As, arsenic; CSF, cancer slope factor; EDI, Estimated Daily Intake; ILCR, Incremental Lifetime Cancer Risk; Pb, lead.

Table 5. Comparison of the EDI of Zn, Fe, Cu and Mg through 10 g tea infusion/day consumption and toxicological parameters.

	Toxicity reference value (mg/day)					Percentage of considered parameters according to EDI			
	PMTDI	RfD	TDI	UL	Average EDI (mg/day)	PMTDI	RfD	TDI	UL
Zn	1		10	40	0.002	0.20		0.02	0.01
Fe	0.8			45	0.008	1.00			0.02
Cu	0.5	0.04		10	0.002	0.40	5.00		0.02
Mg				350	0.026				0.01

Cu, copper; EDI, Estimated Daily Intake; Fe, iron; Mg, magnesium; PMTDI, Provisional TDI; RfD, reference dose; TDI, Tolerable Daily Intake; UL, Tolerable Upper Intake Level; Zn, zinc.

for As, with an estimated maximum limit of 15 µg/kg of BW (equivalent to 900 µg per person; Karak and Bhagat[1]). According to Yuan et al.,[30] the consumption of 10 g of Chinese tea per person daily results in a maximum inorganic As contribution of 2.26 µg from tea infusion. This equates to 0.038 µg/kg/day, excluding the contribution from water. This value reported in that study corresponds to only 1.8% of the recommended PTWI of 2.1 µg per kg of BW per day, as established by the FAO/WHO. In the present study, the consumption of infusions from tea samples in the Turkish market at an IR of 10 g Pbs to an inorganic As intake of 0.025 µg/kg/day. This corresponds to a PTWI that is slightly lower (by 1.2%) than that reported in the study conducted in China.

Exposure to Pb in children continues to be a significant health concern due to the neurological effects that persist from prolonged exposure to relatively low levels of Pb.[31] As a result, numerous countries have established permissible limits for Pb in foods and beverages, acknowledging that these are significant sources of Pb accumulation in the human body. The permissible limit of Pb for food and beverages in Europe is 5 mg/kg[1]. As indicated in Table 1, the concentration of Pb in all investigated tea leaf samples exceeded the limit of 5 mg/kg. However, the levels of Pb in tea infusions were found to be significantly below this limit. None of the individual THQs for Al, As, or Cd exceeded 1, indicating that consumption of the analyzed tea infusions is unlikely to pose significant non-carcinogenic health risks. The HI also remained below 1, suggesting that combined exposure to multiple toxic elements does not result in cumulative adverse effects. Al contributed the largest proportion of the HI, followed by As and Pb, reflecting their relative impact on total risk.

Potential carcinogenic risks from As and Pb exposure through tea consumption were also evaluated in this study. The carcinogenic risk assessment was based on the CSF values for inorganic As (1.5 per mg/kg/day) and Pb (0.0085 mg/kg/day). The CSF is a key toxicity metric that quantifies the relationship between dose and response. ILCR above 10^{-4} suggests a high cancer risk, while an ILCR below 10^{-6} indicates minimal risk. Values falling between 10^{-6} and 10^{-4} denote moderate risk.[9,28] In this study, the ILCR for Pb in all tea infusion samples was below the safe threshold of 10^{-6}, indicating no significant cancer risk. For As, ILCR values ranged from 10^{-6} to 10^{-4} across all samples, indicating a moderate carcinogenic risk. However, the As concentrations measured in this study represent total As, not solely inorganic As. Therefore, the carcinogenic risk might be overestimated because not all As in the samples is present as inorganic species.

For essential elements, the EDI of Fe, Cu, Zn, and Mg from tea infusions was compared to their toxicological reference values, including PMTDI, TDI, UL, or RfD.[32]

Tea is one of the most widely consumed beverages worldwide and represents an important component of a balanced diet. However, tea consumption may also contribute to dietary exposure to both essential and toxic elements because tea plants naturally accumulate them. The analysis of infusions from eight commercially available tea brands indicated that there were no significant non-carcinogenic health risks associated with Al, As, Cd, and Pb, either individually or cumulatively, as reflected by the HI. Although a moderate carcinogenic risk was observed for As, this assessment was based on total As concentrations and may therefore overestimate the actual risk associated with inorganic As.

Continuous monitoring of levels of both toxic and essential elements in foods and beverages remains crucial for quality control and food safety. The findings of this study provide useful data to support ongoing efforts aimed at improving the safety and quality of black tea products in the region.

Study limitations

A limitation of the present study is the relatively small number of tea brands analyzed (n = 8), which may limit statistical power and the generalizability of the findings. Although the selected samples represent some of the most widely consumed black tea products available in the Turkish market, the results should be interpreted with caution and may not fully reflect the variability of all black tea brands and production batches. Future studies that include a larger number of samples from different production origins would be valuable for further refining population-level exposure assessments.

In addition, polyphenols, such as tannins in tea, can chelate metal ions, and divalent metals often form insoluble complexes, both of which can significantly reduce metal bioavailability. However, data on the potential absorption of tannin-metal complexes in tea drinkers are scarce in the literature. Therefore, simply listing metal concentrations is insufficient for a comprehensive risk assessment.

Another limitation of this study is that metal extraction was evaluated at a single brewing time (5 minutes). Although this duration reflects standard preparation practices, variations in brewing time, water temperature, or repeated infusions may influence metal extraction rates. Therefore, the results may not fully represent individual consumption habits, and future studies that assess different brewing conditions would provide a more comprehensive exposure assessment.

CONCLUSION

This study provides an in-depth analysis of the concentrations of essential and toxic elements in black tea bag samples from Türkiye, offering valuable insights into their potential health implications. The findings indicate that both essential and toxic elements are present in varying concentrations in the tea leaves and their infusions. Among the toxic elements, Al and Pb were the most abundant in dry tea leaves, with Al also being the predominant toxic element in tea infusions. Essential elements such as Mg, Fe, Cu, and Zn were also detected, with Mg being the most prevalent.

The health risk assessments (EDI, THQ, and HI) revealed that the levels of individual toxic elements in tea infusions and their cumulative non-carcinogenic risks are within acceptable limits. The carcinogenic risk assessment highlighted a moderate risk for as; however, this is likely overestimated because total,

rather than inorganic, As was measured. Importantly, the essential elements were present at concentrations significantly below their toxicological thresholds, indicating no risk of adverse health effects from consumption of tea infusions. The study underscores the influence of environmental factors, soil characteristics, and agricultural practices on the accumulation of elements in tea plants. It also highlights the importance of extraction efficiencies in determining the transfer of these elements from tea leaves to infusions, with most elements categorized as poorly or moderately extractable under standard brewing conditions.

Ethics

Ethics Committee Approval: Ethical approval is not required for this study.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: M.H., M.Ş., G.E., S.H., A.A., Design: M.H., M.Ş., G.E., S.H., A.A., Data Collection or Processing: M.H., M.Ş., G.E., S.H., A.A., Analysis or Interpretation: M.H., M.Ş., G.E., S.H., A.A., Literature Search: M.H., M.Ş., G.E., S.H., A.A., Writing: M.H., M.Ş., G.E., S.H., A.A.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Karak T, Bhagat RM. Trace elements in tea leaves, made tea and tea infusion: a review. Food Res Int. 2010;43:2234-2252.
2. Chung FL, Schwartz J, Herzog CR, Yang YM. Tea and cancer prevention: studies in animals and humans. J Nutr. 2003;133:3268S-3274S.
3. Kumar A, Nair AGC, Reddy AVR, Garg AN. Availability of essential elements in Indian and US tea brands. Food Chem. 2005;89:441-448.
4. Peycheva K, Panayotova V, Stancheva R, Makedonski L, Merdzhanova A, Parrino V, Nava V, Cicero N, Fazio F. Risk assessment of essential and toxic elements in freshwater fish species from lakes near Black Sea, Bulgaria. Toxics. 2022;10:675.
5. Fernandez PL, Pablos F, Martin MJ, Gonzalez AG. Multi-element analysis of tea beverages by inductively coupled plasma atomic emission spectrometry. Food Chem. 2002;76:483-489.
6. Matsuura H, Hokura A, Katsuki F, Itoh A, Haraguchi H. Multielement determination and speciation of major-to-trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. Anal Sci. 2001;17:391-398.
7. Sultana S, Ahmed FT, Rahman N, Alam MF. Determination of Ni, Cu, Cd, Zn, Pb, Cr and Mn in some black and green tea leaves and their infusions available in Bangladeshi local markets. Appl Chem Eng. 2023;6:28-37.
8. Baldelli A, Arrieta AL, Pratap-Singh A. Inhibition of metal-polyphenol complex in tea fortified with encapsulated iron. Food Bioprocess Technol. 2025;18:1808-1820.
9. Charehsaz M, Helvacıoğlu S, Çetinkaya S, Demir R, Erdem O, Aydin A. Heavy metal and essential elements in beers from Turkey market: a risk assessment study. Hum Exp Toxicol. 2021;40:1241-1249.
10. Helvacıoğlu S, Charehsaz M, Güzelmeriç E, Türköz Acar E, Yeşilada E, Aydın A. Comparative investigation of grape molasses produced by conventional and industrial techniques. Marmara Pharm J. 2018;22:44-51.
11. Karami H, Shariatifar N, Nazmara S, Moazzen M, Mahmoodi B, Mousavi Khaneghah A. The concentration and probabilistic health risk of potentially toxic elements in edible mushrooms (wild and cultivated) samples collected from different cities of Iran. Biol Trace Elem Res. 2021;199:389-400.
12. Czarniecka-Skubina E, Korzeniowska-Ginter R, Pielak M, Sałek P, Owczarek T, Kozak A. Consumer choices and habits related to tea consumption by Poles. Foods. 2022;11:2873.
13. Antoine JMR, Fung LAH, Grant CN. Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicol Rep. 2017;4:181-187.
14. US Environmental Protection Agency. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F. Washington (DC): US EPA; 2002.
15. Tang DS, Shen SR, Chen X, Zhang YY, Xu CY. Interaction of catechins with aluminum *in vitro*. J Zhejiang Univ Sci A. 2004;5:668-675.
16. Zhang J, Yang R, Chen R, Peng Y, Wen X, Gao L. Accumulation of heavy metals in tea leaves and potential health risk assessment: a case study from Puan County, Guizhou Province, China. Int J Environ Res Public Health. 2018;15:133.
17. Ghale-Askari S, Oskoei V, Abedi F, Motahhari-Far P, Naimabadi A, Javan S. Evaluation of heavy metal concentrations in black tea and infusions in Neyshabur city and estimating health risk to consumers. Int J Environ Anal Chem. 2020;102:7928-7937.
18. Pourramezani F, Akrami Mohajeri F, Salmani MH, Dehghani Tafti A, Khalili Sadrabad E. Evaluation of heavy metal concentration in imported black tea in Iran and consumer risk assessments. Food Sci Nutr. 2019;7:4021-4026.
19. Al-Oud SS. Heavy metal contents in tea and herb leaves. Pak J Biol Sci. 2003;6:208-212.
20. Rahmani A, Kalteh S, Derakhshan-Jazari M, Baziar M, Karimaei M. Trace elements in imported black tea products in Iran: quantification and probabilistic health risk assessment. Int J Environ Anal Chem. 2022;104:2691-2706.
21. Ozdemir Z, Zannou O, Koca I. Assessment of the aluminium contents of black tea and black tea infusions. Discov Food. 2022;2:13.
22. Nour SMF, El-Desoky AMM, Hassan NA, Osman KA. Estimated daily intake of epichlorohydrin and certain heavy metals of bagged and loose black teas. J Food Sci Technol. 2023;60:666-678.
23. Yaylalı-Abanuz G, Tüysüz N. Heavy metal contamination of soils and tea plants in the eastern Black Sea region, NE Turkey. Environ Earth Sci. 2009;59:131-144.
24. Özyazıcı MA, Dengiz O, Aydoğan M. Reaction changing and distribution of agricultural land for tea cultivation. Soil Water J. 2013;2:23-29.
25. Salahinejad M, Aflaki F. Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. Biol Trace Elem Res. 2010;134:109-117.
26. Costa LM, Gouveia ST, Nóbrega JA. Comparison of heating extraction procedures for Al, Ca, Mg and Mn in tea samples. Anal Sci. 2002;18:313-318.
27. Natesan S, Ranganathan V. Content of various elements in different parts of the tea plant and in infusion of black tea from South India. J Sci Food Agric. 1990;51:125-139.

28. US Environmental Protection Agency. Risk assessment guidance for superfund. Volume I: human health evaluation manual (Part E, supplemental guidance for dermal risk assessment). EPA/540/R/99/005. Washington (DC): US EPA; 2004.

29. Bondy SC. The neurotoxicity of environmental aluminum is still an issue. Neurotoxicology. 2010;31:575-581.

30. Yuan C, Gao E, He B, Jiang G. Arsenic species and leaching characters in tea (Camellia sinensis). Food Chem Toxicol. 2007;45:2381-2389.

31. Helvacıoğlu S, Charehsaz M, Erdem O, Aydın A. Assessment of toxic element content of some grape molasses produced by conventional and industrial techniques: insights into human safety. Toxin Rev. 2019;40:1198-1205.

32. Olmedo P, Hernández AF, Pla A, Femia P, Navas-Acien A, Gil F. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples: risk and nutritional assessment and mercury-selenium balance. Food Chem Toxicol. 2013;62:299-307.

Turk J Pharm Sci 2026;23(1):31-40
DOI: 10.4274/tjps.galenos.2026.80025

ORIGINAL ARTICLE

Tannic Acid and Copper-Modified ZIF-8 Metal Organic Framework as a Cefotaxime Delivery System for Antimicrobial Activity

Yağmur PİRİNÇÇİ TOK[1]*, Münteha ÖZSOY[2], Damla DAMAR ÇELİK[3]

[1]İstanbul Health and Technology University Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye
[2]Kocaeli Health and Technology University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Kocaeli, Türkiye
[3]Marmara University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, İstanbul, Türkiye

ABSTRACT

Objectives: Antibiotic resistance has become a global public health threat. Cefotaxime (CTX), a third-generation cephalosporin, is approved for use in infants, children, and adults with various microbial infections, particularly those affecting the central nervous system. This study aimed to synthesize and characterize zeolitic imidazolate framework (ZIF)-8-based drug delivery systems (DDSs) to enhance antimicrobial activity and control CTX release.

Materials and Methods: ZIF-8 was synthesized via the coordination network of Zn ions and 2-methylimidazole and subsequently modified with tannic acid (TA) and copper ions (Cu^{2+}). ZIF-8 MOF and its derivatives were characterized by Fourier Transform Infrared, zeta potential, *in vitro* dissolution rate, and *in vitro* antimicrobial activity.

Results: The drug loading capacity and encapsulation efficiency were found to be 39.50 ± 1.19% and 98.75 ± 2.96%, respectively, for ZIF-8@TA@CTX, and 40.75 ± 1.22% and 97.75 ± 2.93%, respectively, for ZIF-8@TA@Cu@CTX. Following 48 hours, the drug released from ZIF-8@TA@Cu@CTX was detected at 62.83 ± 1.89% at pH 5.0 and 83.19 ± 2.50% at pH 7.4 after 48 h, with dissolution profiles best fitting the Korsmeyer–Peppas model. The synthesized DDSs demonstrated a higher antibacterial activity against gram-positive bacteria than against gram-negative bacteria.

Conclusion: ZIF-8 MOF DDS may serve as an alternative for delivering drugs to infected areas due to their controlled release under low pH conditions.

Keywords: Antibiotic resistance, metal organic framework, zeolitic imidazolate frameworks, cefotaxime

INTRODUCTION

Antibiotic resistance has become a global public health burden.[1] Each year, 700.000 people worldwide lose their lives due to antibiotic resistance, and this critical issue is predicted to cause more than 10 million annual deaths by 2050.[2] In addition to the misuse and overuse of antibiotics in humans, antibiotic resistance can occur naturally, through hereditary changes in bacterial strains, or via the transfer of resistant genes between bacteria.[3,4]

Cefotaxime (CTX) is a member of the third-generation cephalosporin group of antibiotics with greater activity against both gram-negative and gram-positive bacteria than the first and second generations.[5] It exhibits activity by preventing the biosynthesis of peptidoglycan, which is involved in the formation of the bacterial cell wall.[6] The Food and Drug Administration has approved its use for infants, children, and adults with various microbial infections, including those affecting the central nervous system, lower respiratory tract, bone and joint, genitourinary, and skin systems.[7,8] Specifically, the World Health Organization has offered it as an essential medicine for treating life-threatening meningitis.[9] However, extended-spectrum β-lactamase has been reported as a

***Correspondence:** yagmur.tok@istun.edu.tr, **ORCID-ID:** orcid.org/0000-0001-6915-0283
Received: 13.09.2025 , **Accepted:** 05.03.2026 **Epub:** 10.04.2026 **Publication Date:** 13.07.2026

Cite this article as: Pirinççi Tok Y, Özsoy M, Damar Çelik D. Tannic acid and copper-modified ZIF-8 metal organic framework as a cefotaxime delivery system for antimicrobial activity. Turk J Pharm Sci. 2026;23(1):31-40

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

resistance mechanism of bacteria against third-generation cephalosporins (e.g., CTX).[10]

Using nanomaterials as drug delivery systems (DDSs) to combat antibacterial resistance has been of growing interest.[11] A number of strategies based on nanomaterials, including nanoparticles and nanosystems based on metals and carbon, have been preferred to overcome the challenges of current antibiotic therapies[12] due to their large surface area, enhanced intracellular penetration, and multidrug combination.[13] Furthermore, surface modification of nanosystems enables them to target the infected site, thereby enhancing cell internalization and minimizing systemic exposure.[14]

Metal-organic frameworks (MOFs) are highly porous coordination systems comprising metal-ion nodes and organic ligands.[15] Their high surface-to-volume ratio, greater drug loading (DL) capacity, tunable surfaces, and biocompatible properties make them attractive DDSs.[16] Recently, MOFs have been applied in the literature for advanced antibacterial therapy, and this success can be attributed to different strategies. The MOF structure can consist of different metal ions, such as zinc, silver, iron, and copper. Therefore, bacterial growth can be prevented due to the antibacterial properties of these ions.[17] Furthermore, organic ligands, such as porphyrins, show photocatalytic activity with the help of light, thus facilitating the death of bacteria by forming reactive oxygen species. Lastly, MOFs can load cargo with many antibacterial activities, including drugs and antimicrobial peptides, which are triggers to kill bacteria.[18]

Zeolitic imidazolate frameworks (ZIFs) are a subclass of MOFs composed of tetrahedrally coordinated metal ions linked by imidazolate ligands. This results in the formation of zeolite-like structures. ZIF-8, constructed from zinc ions (Zn^{2+}) and 2-methylimidazolate linkers, is one of the most widely investigated ZIF materials due to its high porosity, structural stability, and pH-responsive degradation behavior, which render it attractive for drug delivery applications.[19] For instance, Costa et al.[2] combined ZIFs and zinc oxide (ZnO) nanoparticles to increase ciprofloxacin activity and provide its controlled release. The DL process was conducted following the incorporation of ZnO with ZIF-8. The resultant complex (CIP-ZnO@ZIF-8) demonstrated significantly elevated activity, exhibiting 10- and 200-fold higher activity against the *Staphylococcus aureus* strain and *Pseudomonas aeruginosa*, respectively.

Tannic acid (TA) is a natural secondary compound found in numerous plants and has various pharmacological activities, including anti-tumor, anti-diabetes, anti-obesity, and anti-myocardial ischemia.[20] Recently, TA has been encapsulated in different nanoscale DDSs to increase its antimicrobial (e.g., antiviral, antibacterial, and antifungal) and anti-inflammatory activities.[21]

In 2008, the Environmental Protection Agency approved copper (Cu^{2+} ions) as the pioneer metallic antimicrobial agent, which can kill bacteria at a rate of 99.9%.[22] Copper ions (Cu) penetrate the bacterial cell membrane, enter the cell via transport proteins following membrane rupture, and bind to essential components such as DNA and proteins. This results in cell death and antibacterial activity.[23]

The current study aimed to investigate the efficacy of ZIF-8 (ZIF-8@TA@Cu@CTX), a TA- and Cu-functionalized material, in enhancing the antibacterial activity and controlling the release of CTX. For this purpose, ZIF-8 was synthesized by a one-pot method and functionalized with TA and Cu, which may contribute to a reduction in the dose of CTX due to their inherent antibacterial properties. The synthesis of ZIF-8 was confirmed by X-ray diffraction, thermogravimetric analysis, and scanning electron microscopy, and the structure of its derivatives was verified by Fourier Transform Infrared (FT-IR) analysis. ZIF-8, ZIF-8@TA, ZIF-8@TA@Cu, ZIF-8@TA@CTX, and ZIF-8@TA@Cu@CTX were subjected to *in vitro* antibacterial tests to investigate and compare their antibacterial activity. Finally, the *in vitro* drug release behavior in the dissolution media, namely acetate buffer (pH 5.0) and phosphate buffer (pH 7.4), was evaluated.

MATERIALS AND METHODS

Materials

Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$], 2-methylimidazole (2-MeIm), TA, and copper (II) sulfate pentahydrate ($CuSO_45H_2O$) were obtained from Sigma-Aldrich. Methanol (MeOH) and dimethyl sulfoxide (DMSO) were obtained from MERCK Millipore, Germany. A Sartorius Arium Pro purification system was used to supply ultrapure water.

Synthesis of the ZIF-8

ZIF-8 was produced according to the ratio reported by Özsoy et al.[24] 1.35 g of $Zn(NO_3)_2{\cdot}6H_2O$ and 0.75 g of 2-MeIm were dissolved in 30 mL of MeOH separately. The latter solution was added dropwise to the zinc nitrate solution, and the mixture was stirred for 1 h. Finally, ZIF-8 complexes were obtained by washing with MeOH after for centrifugation at 4000 rpm for 15 minutes, and this procedure was performed three times.

Modification of ZIF-8 using TA and Cu

TA and copper sulfate solutions were prepared separately to modify the ZIF-8 surface; 0.5 g of TA was dissolved in deionized distilled water (DDW), and 0.2 g of ZIF-8 was dispersed in MeOH. TA solution was added dropwise to the ZIF-8 dispersion to form ZIF-8@TA, and stirring was continued for 2 h. The mixture was centrifuged at 4000 rpm for 15 min and washed with MeOH to obtain the final product (ZIF-8@TA). This process was repeated three times. ZIF-8@TA was dispersed in MeOH, and copper sulfate solution (0.2 g in DDW) was added dropwise to synthesize ZIF-8@TA@Cu. The same centrifugation and washing procedures described above were applied.

Synthesis of ZIF-8@TA@CTX and ZIF-8@TA@Cu@CTX

The drug-to-delivery system ratio was adjusted to 1:2.5.[24] The CTX solution in DMSO was added dropwise to the ZIF-8@TA and ZIF-8@TA@Cu dispersions separately for each dispersion. The necessary steps to obtain the final products were performed following a 1-day stirring period at room temperature, as mentioned above.

DLC and EC of ZIF-8@TA@Cu@CTX

The amount of CTX loaded into the designed DDSs was determined using the standard curve at 260 nm ultraviolet–visible (UV–Vis) spectroscopy.[8] Equations (1) and (2) were used to calculate the CTX loading capacity (%) and EE (%).[25]

$$\text{DL capacity (\%)} = \frac{\text{Amount of drug in carrier (mg)}}{\text{Amount of carrier (mg)}} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug in carrier (mg)}}{\text{Amount of drug added initially}} \times 100 \quad (2)$$

In vitro dissolution rate studies from designed DDS

To evaluate the drug release behavior of the designed ZIF-8@TA@Cu@CTX, *in vitro* dissolution rate studies were conducted in two distinct media (acetate, pH 5.0, and phosphate buffer, pH 7.4). ZIF-8@TA@Cu@CTX was selected as a representative formulation for dissolution kinetics studies because the incorporation of Cu^{2+} was anticipated a priori to have the greatest impact on both the coordination environment and release behavior. A specific quantity of sample used in drug release studies was dispersed individually in 10 mL of pH 5.0 and pH 7.4 buffers via sonication and subsequently sealed within dialysis bags. These bags were then immersed individually in 50 mL of the corresponding pH buffer and agitated at 37 °C using a magnetic stirrer set to 200 rpm for a duration of 48 hours. At periodic intervals, 1 mL of the sample was taken for analysis and replaced with a fresh buffer solution to ensure the maintenance of sink conditions. The drug amounts in the samples collected from the release medium were determined using UV-Vis at 260 nm wavelength. Following the determination of the drug's release at the point of interest, numerous mathematical models, such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas have been used to evaluate the release profiles. The equations for each model are given below. The parameter k represents each model's rate constant, n indicates the release exponent, and Q represents the amount of drug dissolved at time t.[26]

$$\begin{aligned} Q_t &= k_0 \cdot t \\ Q_t &= Q_0 \cdot (1 - e^{-k_1 \cdot t}) \\ Q_t &= k_H \cdot \sqrt{t} \\ Q_t &= k \cdot t^n \end{aligned} \quad (3)$$

Characterization studies

To identify the functional groups and determine the structural characterization of the ZIF-8, ZIF-8@TA, ZIF-8@TA@Cu, ZIF-8@TA@CTX, and ZIF-8@TA@Cu@CTX samples, FT-IR analysis was conducted on a Perkin Elmer instrument in the range of 4000–400 cm^{-1}. Characteristic peaks were evaluated from the obtained spectra, and the chemical structure was confirmed.

The zeta potential, particle size, and polydispersity index (PDI) of rehydrated samples were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Zeta potential measurements were performed after rehydration in buffer solutions at a defined concentration, whereas particle size measurements were conducted following rehydration in deionized water.[27]

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

This assay involves determining the antimicrobial susceptibility spectrum of compounds according to the resistance of gram-positive and gram-negative bacteria using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. The MIC is the lowest concentration of molecules that completely inhibits visible growth. The antimicrobial properties of the solvents were assessed to serve as a control, and the results of the tests were evaluated in accordance with established protocols.[28] Bacterial inocula were prepared from overnight cultures to achieve concentrations of 1 x 10^8 colony forming units (CFU)/mL. Mueller–Hinton broth (Oxoid) was used to determine the MIC, while tryptic soy agar (TSA, Difco Laboratories) was used to evaluate the MBC value.

The MBC refers to the lowest concentration of antimicrobial agents required to attain a 99.9% reduction in the initial inoculum, as quantified by CFU counts. 10 µL samples were extracted from each well of the microplate and transferred to TSA-containing Petri dishes. After a 24-h incubation period at 37 °C, the colonies were inspected to ascertain the presence of MBC.[29] The positive control wells contained bacteria without any drugs, and the negative control wells contained only the media and were used to confirm the sterility of the medium. The tests were performed using standard antibacterial agents, such as meropenem and ciprofloxacin, as controls.[28]

Statistical analysis

The data obtained from *the in vitro* dissolution studies were statistically analyzed through non-linear regression using the GraphPad Prism 9.0 statistical program. The microbiological results were interpreted in accordance with the sensitivity limits established by recognized authorities, such as the CLSI or the European Committee on Antimicrobial Susceptibility Testing. Because MIC is not a continuous variable, statistical analysis is not required for MIC testing. All characterization tests except for FT-IR analysis were performed in triplicate (n = 3). Standard deviations presented where applicable. In the antimicrobial tests, standard deviations were not reported when identical concentration values were obtained across replicates. This information has been clarified in the revised manuscript.

RESULTS

ZIF-8 was synthesized via coordination polymerization and modified with TA and Cu, respectively. After these modifications, CTX was separately loaded on ZIF-8@TA and ZIF-8@TA@Cu to evaluate their antimicrobial activity as DDSs, as shown in Figure 1.

In the ZIF-8 spectrum (Figure 2), the first stretching peak at 3200 cm^{-1} was due to the N-H bond, and the other small peaks at 3100 and 2800 cm^{-1} could be attributed to the imidazole ring and methyl group, respectively.[30] The peaks at 1450 and 1560 cm^{-1} were determined to be due to the C=N stretching modes from 2-Melm, whereas the peak at 1380 cm^{-1} was attributed to the entire ring stretching. The stretching peak that appeared

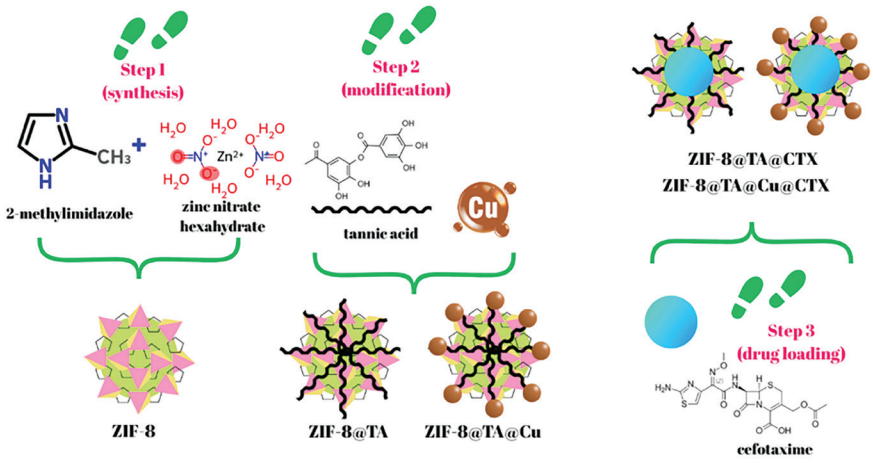


Figure 1. Synthesis of ZIF-8@TA, ZIF-8@TA@Cu, ZIF-8@TA@CTX, and ZIF-8@TA@Cu@CTX.
CTX, cefotaxime; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

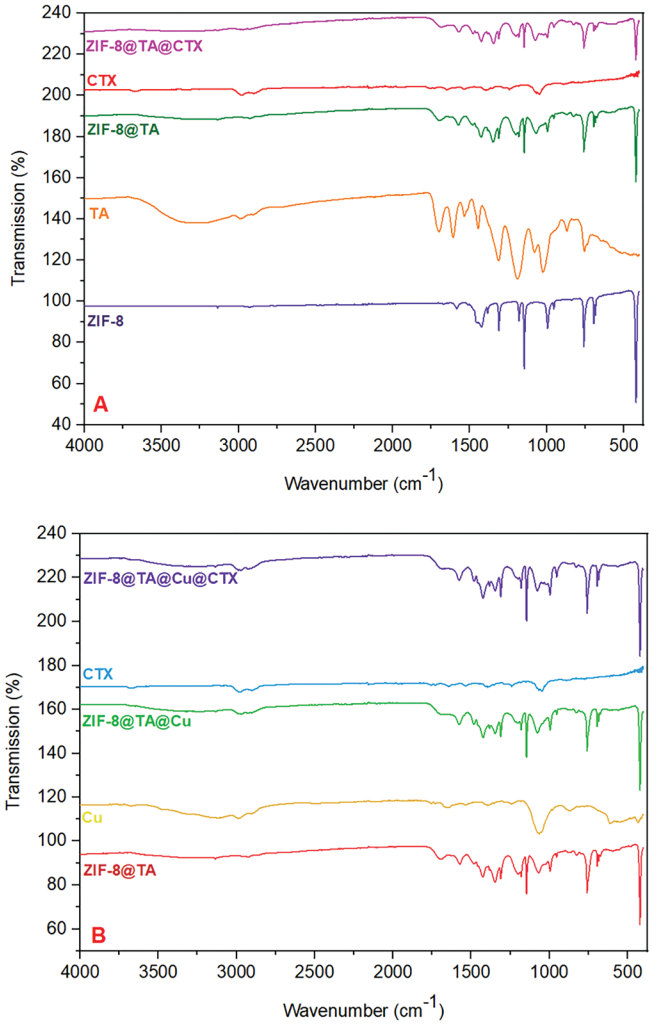


Figure 2. FT-IR spectra of the synthesized DDSs A (ZIF-8@TA@CTX synthesis steps) and B (ZIF-8@TA@Cu@CTX synthesis steps).
CTX, cefotaxime; DDSs, drug delivery systems; FT-IR, Fourier Transform Infrared; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

at 1000 cm^{-1} was due to C-N bond of imidazole, and the peaks ranging from 1100 to 900 cm^{-1} could be related to the in-plane bending of the ring. The peak observed at 420 cm^{-1} confirmed the formation of the ZIF-8 structure due to the Zn-N bond formed as a result of the reaction between 2-MeIm and Zn atoms.[24] In the TA spectrum, a broad band ranging from 3500–3200 cm^{-1} was attributed to the stretching vibration of O-H groups, while the peaks at 2900–2800 cm^{-1} were associated with C-H stretching vibrations of CH_2 and CH_3, respectively. The stretching vibrations of C=O from the carbonyl group located at 1700 cm^{-1}, which confirms aromatic ester moieties.[31] The ZIF-8@TA spectrum show broad O–H (≈3300 cm^{-1}) and C–O (≈1210/1040 cm^{-1}) bands characteristic of TA. The C=O band of TA was weaker and shifted ~20 cm^{-1} downwards relative to TA, and a shift of around 4 cm^{-1} was observed in the Zn–N band. These findings indicate that the phenolic groups were bound via Zn^{+2} surface coordination/H-bonding. The appearance of β-lactam C=O (~1700 cm^{-1}), amide I (~1600 cm^{-1}) and amide II (~1560 cm^{-1}) bands specific to CTX in the spectrum of ZIF-8@TA@CTX, the displacement of C–O bands of TA (≈1210/1040 cm^{-1}) and the preservation of ZIF-8 backbone bands (and ~420 cm^{-1}) indicate that CTX was successfully immobilized on ZIF-8@TA. The spectrum of ZIF-8@TA@Cu showed a decrease in the width of the TA O–H band and a downward shift of the C–O bands (≈1210/1040 cm^{-1}) by ≈12 cm^{-1}, as well as the appearance of a new, weak Cu–O band around 615 cm^{-1} and the preservation of the ZIF-8 skeleton bands (e.g. ≈420 cm^{-1}), indicate that Cu^{2+} is coordinatively bound to the surface via TA. Similar to ZIF-8@TA@CTX, characteristic peaks belonging to CTX were also observed in the ZIF-8@TA@Cu@CTX spectrum.

The surface charges of ZIF-8 and its derivatives were examined by zeta potential measurement, as shown in Figure 3. ZIF-8 exhibited a negative surface charge in acetate buffer (pH 5.0) and phosphate buffer (pH 7.4), in line with the literature.[32] The analysis of particle size and PDI performed on rehydrated formulations revealed hydrodynamic diameters in the micron range (2380 ± 209 nm and PDI: 0.24 ± 0.04 for ZIF-8@TA@

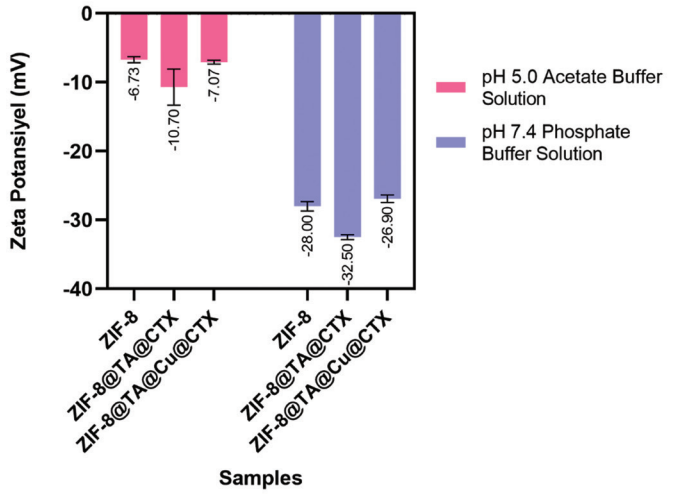


Figure 3. Zeta potential of ZIF-8 MOF and its derivatives in aqueous buffer solutions.

CTX, cefotaxime; MOF, metal-organic frameworks; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

CTX and 3223 ± 293 nm and PDI: 0.36 ± 0.09 for ZIF-8@TA@Cu@CTX). Secondary aggregates may influence mass transfer and biological interactions, as suggested by micron-scale hydrodynamic diameters.

Loading capacity and encapsulation efficacy of CTX

CTX loading in ZIF-8 MOFs was measured using an indirect method. The EE and DLC were determined using a standard curve with the range of 3.96–23.76 µg/mL, as illustrated in Figure 4. The limit of detection and limit of quantification of the method were 1.10 and 3.33 µg/mL, respectively. The DLC and EE were detected as 39.50 ± 1.19% and 98.75 ± 2.96% for ZIF-8@TA@CTX, respectively, while they were 40.75 ± 1.22% and 97.75 ± 2.93% for ZIF-8@TA@Cu@CTX, respectively (Figure 5).

Determination of MIC and MBC

The *in vitro* antimicrobial activity of the DDSs against five gram-negative bacteria and four gram-positive bacteria determined by the dilution technique using CLSI recommendations. Table 1 presents the results of the antimicrobial experiments for all samples.

The test cultures, *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853 and *Escherichia coli* ATCC 25922, demonstrated resistance to all synthesized ZIF-8 MOFs. Among the DDSs tested, only ZIF-8@TA@Cu showed antimicrobial activity against both Proteus vulgaris ATCC 13315 and methicillin-resistant *S. aureus* (MRSA) ATCC 43300 with MIC values of 1250 µg/mL and 312.5 µg/mL, respectively. ZIF-8@TA@Cu@CTX had the lowest MIC activity with 312.5 µg/mL against *Klebsiella pneumoniae* ATCC 4352 among gram-negative bacteria.

All DDSs except for ZIF-8 showed good activity against *S. aureus*, with an MIC value of 39.06 (DDSs except for ZIF-8 and last one in Table 1) and 19.53 g/mL (for ZIF-8@TA@Cu@CTX) 19.53 µg/mL. Besides, ZIF-8@TA@CTX, and ZIF-8@TA@Cu@CTX had the same inhibitory activity against *Staphylococcus epidermidis* ATCC 12228 with an MIC value of 19.53 µg/mL.

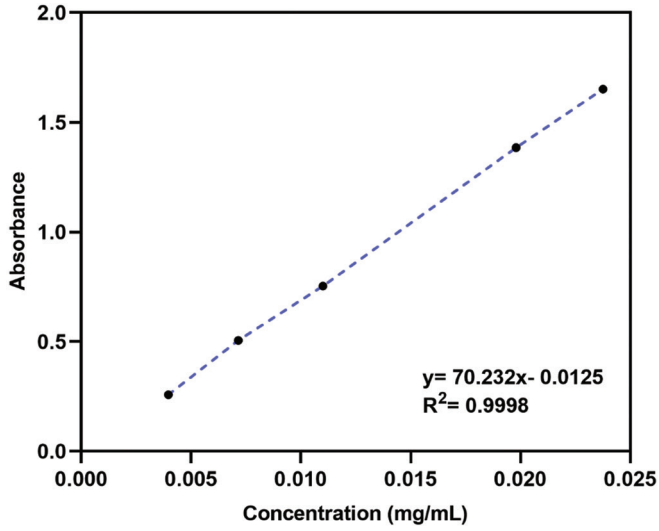


Figure 4. Standard curve of CTX for EE and DL evaluation.

CTX, cefotaxime; DL, drug loading; EE, encapsulation efficiency.

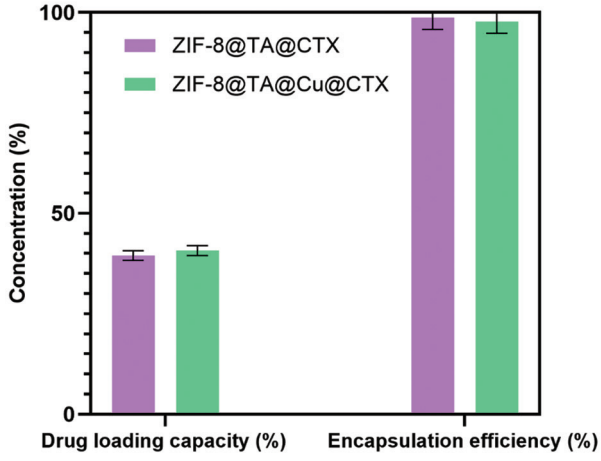


Figure 5. Concentration of DL and EE for CTX.

CTX, cefotaxime; DL, drug loading; EE, encapsulation efficiency; CU, copper ion.

The MBC values of the DDSs were found against *S. aureus* ATCC, *S. epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 29212 among Gram-positive bacteria, and ZIF-8, ZIF-8@TA, and ZIF-8@TA@Cu@CTX exhibited the lowest MBC values against *S. epidermidis* ATCC 12228 (Table 2).

The drug release profile of ZIF-8@TA@Cu@CTX in acetate (pH 5.0) and phosphate buffer (pH 7.4) solutions at 37 ± 0.5° C is shown in Figure 6. The quantity of drug released was calculated based on the analytical curve. Within the first six hours, CTX release from ZIF-8@TA@Cu@CTX was observed to be 25.78 ± 0.77% in a pH 5.0 acetate buffer, while the cumulative drug release was determined to be 71.64 ± 2.15% in a pH 7.4 phosphate buffer. Following a 48-hour period, the percentage of drug release was 62.83 ± 1.89% at pH 5.0 and 83.19 ± 2.50% at pH 7.4.

The release pattern data were processed using GraphPad Prism software to analyze the mechanism of drug release

kinetics. The parameters of each model are shown in Table 3. The *in vitro* dissolution rate results were best fitted Korsmeyer–Peppas kinetics model (Figure 7), which yielded the highest R^2 value in both dissolution media.

DISCUSSION

Recently, there has been a mounting interest in the use of MOF-based DDSs as a means of combating antibiotic resistance. ZIF-8 offers a highly porous delivery system and has antibacterial and anti-inflammatory properties due to the presence of Zn^{+2} ions in its composition.[33] For example, Costa et al.[2] combined ZnO nanoparticles with ZIF-8 and loaded them with the antibiotic ciprofloxacin to increase their antimicrobial activity through synergistic efficacy. They concluded that the combination of CIP and ZnO@ZIF-8 reduced the amount of CIP required to achieve an MIC similar to that of pure ciprofloxacin.[2]

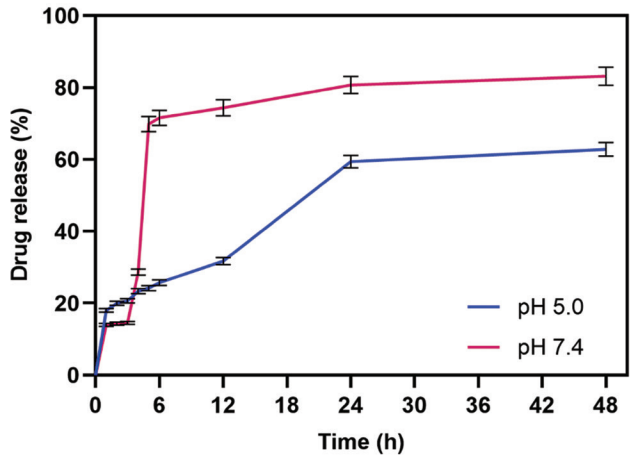


Figure 6. Cumulative release of CTX from ZIF-8@TA@Cu@CTX in acetate (pH 5.0) and phosphate buffer (pH 7.4) solutions.

CTX, cefotaxime; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

Table 1. MIC (mg/L) of synthesized DDSs against gram-negative and gram-positive bacteria.

Synthesized DDSs	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 4352	*P. vulgaris* ATCC 13315	*E. faecalis* ATCC 29212	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 29213	*A. baumannii* ATCC 19606	MRSA ATCC 43300
ZIF-8	-	-	-	-	625	39.06	156.25	625	-
ZIF-8@TA	-	-	625	-	625	39.06	39.06	625	-
ZIF-8@TA @Cu	-	-	625	1250	625	39.06	39.06	625	312.5
ZIF-8@TA @CTX	-	-	625	-	625	19.53	39.06	625	-
ZIF-8@TA @Cu@CTX	-	-	312.5	-	156.25	19.53	19.53	625	-
CTX	8	0.125	0.060	16	1	1	1	16	4
Reference	0.5[a]	0.06[a]	0.5[a]	0.125[a]	2[a]	0.25[a]	0.06[a]	0.5[a]	1[b]

[a]Meropenem; [b]Ciprofloxacin. ATCC, American Type Culture Collection; CTX, cefotaxime; DDSs, drug delivery systems; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

Table 2. MBC (mg/L) of synthesized DDSs against gram-negative and gram-positive bacteria.

Synthesized DDSs	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 4352	*P. vulgaris* ATCC 13315	*E. faecalis* ATCC 29212	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 29213	A. baumannii ATCC 19606	MRSA ATCC 43300
ZIF-8	-	-	-	-	625	39.06	625	-	-
ZIF-8@TA	-	-	-	-	625	78.12	1250	-	-
ZIF-8 @TA @Cu	-	-	-	1250	625	312.5	625	-	-
ZIF-8 @ TA @ CTX	-	-	-	-	625	312.5	1250	-	-
ZIF-8 @TA @Cu@CTX	-	-	-	-	156.25	19.53	1250	-	-
CTX	8	0.6	0.06	16	1	1	2	32	8
Reference	0.5[a]	0.06[a]	0.5[a]	0.125[a]	2[a]	0.25[a]	0.06[a]	0.5[a]	1[b]

[a]Meropenem; [b]Ciprofloxacin. ATCC, American Type Culture Collection; CTX, cefotaxime; DDSs, drug delivery systems; MBC, minimum bactericidal concentration; MRSA, methicillin-resistant *S. aureus*; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

Table 3. Drug release kinetic model parameters for ZIF-8@TA@Cu@CTX.

Sample name	Zero order		First order		Higuchi		Korsmeyer–Peppas	
	R^2	K_0	R^2	K_1	R^2	K_h	R^2	n
pH 5.0	0.848	1.083	0.749	-0.024	0.895	10.31	0.908	0.472
pH 7.4	0.446	32.49	0.366	-0.019	0.580	15.98	0.729	0.614

CTX, cefotaxime; TA, tannic acid; ZIF, zeolitic imidazolate framework; CU, copper ion.

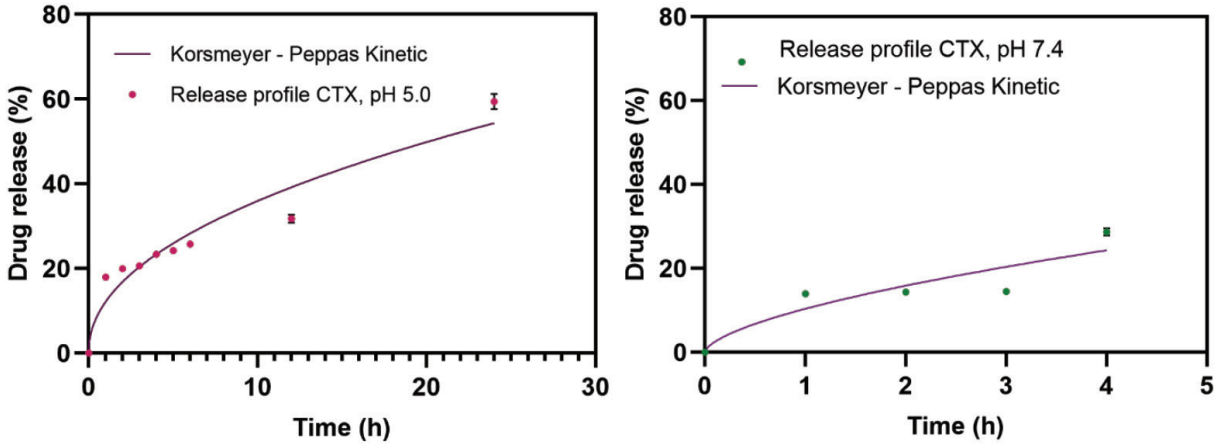


Figure 7. Korsmeyer–Peppas kinetics model fitting curves of DDS in acetate (pH 5.0) and phosphate buffer (pH 7.4) solutions.
CTX, cefotaxime; DDS, drug delivery system.

In this study, ZIF-8 MOF was successfully synthesized and modified with TA and Cu by incorporating antimicrobial CTX. As seen in Figure 2 the formation of ZIF-8 MOF, surface modifications, and DL were confirmed by the functional groups and stretching vibrations in the 4000-400 cm^{-1} spectra. This was further validated using zeta potential measurements. The difference in surface charge illustrated that the compounds (e.g., TA and Cu ions) were successfully loaded on the ZIF-8 MOF surface (Figure 3). The absolute potential increased when TA was bonded to the ZIF-8 surface due to the -OH moieties, whereas it decreased when Cu ions were attached due to their positive charge.

Owing to the large cages of ZIF-8 MOFs,[2] the DLC values (39.50% for ZIF-8@TA@CTX and 40.75% for ZIF-8@TA@Cu@CTX) were quite consistent with the literature.

It was initially anticipated that CTX release would be higher under acidic conditions (pH 5.0 acetate buffer), considering the pH-sensitive nature of ZIF-8 and the protonation-induced weakening of coordination between imidazole ligands and Zn^{2+} ions. Therefore, acidic environments are commonly associated with ZIF-8 degradation and drug release.[34] However, the experimentally observed higher drug release at pH 7.4 phosphate buffer can be explained by the specific role of phosphate ions present in the dissolution medium. As previously reported by Velásquez-Hernández et al.,[35] phosphate species exhibit a strong affinity toward Lewis acidic metal centers such as Zn^{2+}, effectively competing with imidazole ligands and accelerating framework decomposition in PBSs. This ligand-exchange process leads to ZIF-8 structural disruption and promotes rapid drug liberation, accompanied by the formation of insoluble zinc phosphate species.[35] Accordingly, the pronounced burst release observed in the pH 7.4 medium during the initial hours can be attributed to phosphate-induced ZIF-8 degradation rather than to pH effects alone. In contrast, no abrupt framework collapse was observed under acidic but phosphate-free conditions (pH 5.0 acetate buffer), resulting in a more gradual and sustained release profile extending up to 48 h.

In addition to chemical and buffer-dependent factors, the particle size may contribute to the observed release behavior. Measurements of the rehydrated formulations revealed hydrodynamic diameters in the micron range, indicating the formation of secondary aggregates upon re-dispersion. Such aggregation, which can be promoted by multidentate interactions of TA and further enhanced by Cu^{2+}-mediated interparticle bridging, may reduce the available effective surface area for mass transfer.[19,36] Moreover, micron-scale hydrodynamic sizes can limit immediate particle–bacteria contact, which has been reported to contribute to sustained drug release behavior and to restrict the apparent enhancement of antimicrobial potency observed in MIC and MBC assays for nanoparticle-based antibiotic delivery systems.[37,38]

Overall, these findings indicate that drug release from ZIF-8-based DDSs is governed not only by pH sensitivity but also by buffer composition and the rehydrated system's physical state. While the faster release observed at pH 7.4 may be attributed to phosphate-induced framework destabilization, the sustained release under mildly acidic, phosphate-free conditions could result from controlled framework degradation combined with

diffusion through the MOF structure. In addition, the micron-scale hydrodynamic sizes observed after rehydration may contribute to prolonged release by limiting the effective surface area and mass transfer. Taken together, these characteristics support the potential applicability of ZIF-8-based DDSs for infection-associated environments, which are often characterized by local acidosis.[39]

As given in the Korsmeyer–Peppas equation, n denotes the release exponent, where if n ≤0.45, the release is controlled by Fickian diffusion. Values ranging from 0.45 to 0.89 indicate a non-Fickian diffusion process, whereas values greater than 0.89 correspond to case-II transport or zero-order (swelling-driven) release.[40] n is a useful indicator to determine the release mechanism. The release exponent increased from 0.472 at pH 5.0 to 0.614 at pH 7.4 when the Korsmeyer–Peppas model was applied to the initial release region of ≤60%. The value at pH 5.0 indicates a diffusion-dominated early release with minor deviations from ideal Fickian behavior, possibly resulting from drug desorption from the ZIF-8 cages and subsequent CTX diffusion through the MOF structure. Conversely, higher n values at pH 7.4 indicate anomalous transport, which is consistent with the contribution of phosphate-induced framework destabilization in addition to diffusion.[41] Therefore, it appears that the early release of CTX in phosphate buffer is governed by coupled diffusion and matrix disruption processes, whereas a more diffusion-controlled profile is maintained under acidic, phosphate-free conditions.

The CTX release profile revealed a pronounced initial release, particularly at physiological pH, followed by a sustained release phase. Although such release behavior could be expected to support antibacterial activity, the MIC or MBC values of the CTX-loaded DDSs were not lower than those of free CTX. This outcome suggests that antibacterial performance is influenced by factors beyond the overall amount of drug released.[42] Despite the inherent antibacterial properties of TA and Cu^{2+} ions, their incorporation into ZIF-8 predominantly results in their coordination or surface association, thereby constraining their direct and independent interaction with bacterial cells under MIC and MBC testing conditions. Although CTX was released in substantial amounts as detected by the analytical method, transient interactions with Zn^{2+}/Cu^{2+} ions and TA may reduce the fraction of freely available and biologically active drug during early exposure.[43] In parallel, non-covalent interactions between CTX and polyphenolic compounds, such as TA, may transiently influence the fraction of pharmacologically active drugs during early exposure.

Furthermore, the local microenvironment generated during the partial degradation of the ZIF-8 framework, including transient metal ion release and local coordination effects, may influence the immediate availability of CTX for bacterial targets rather than causing chemical degradation of the drug. Such effects are of particular relevance to β-lactam antibiotics, whose antibacterial activity depends on the freely accessible fraction of the drug during early exposure rather than on the total drug concentration.[44]

The findings of this study indicate that the ZIF-8-based DDS is not primarily designed to surpass free CTX in short-term *in vitro* assays, but rather to provide controlled delivery and prolonged local exposure. Therefore, the observed antibacterial behavior reflects the balance between release kinetics, drug–carrier interactions, and microenvironmental factors, rather than an unexpected loss of antimicrobial efficacy. It is also important to note that the MIC and MBC assays are based on short-term exposure and, therefore, may not fully capture the potential benefits of SR formulations, especially in the context of localized infection.

From a clinical perspective, the ZIF-8-based DDS was not primarily intended to reduce the systemic dose of CTX but rather to modulate its local availability through controlled release. This approach may be of particular relevance in cases of localized infections, where prolonged exposure at the infection site could enhance antibacterial efficacy. With regard to safety considerations, Cu^{2+} ions were incorporated into the DDS in a coordinated state within the ZIF-8 framework, which may limit the immediate availability of free Cu^{2+} ions under physiological conditions. However, further detailed *in vivo* investigations are required to comprehensively evaluate copper biodistribution, toxicity, and clearance. In conclusion, while the present study provides proof-of-concept *in vitro* findings, further *in vivo* studies are required to more accurately assess the system's translational potential.

CONCLUSION

In this study, a ZIF-8-based MOF DDS was successfully synthesized and further modified with TA and Cu ions. The high porous nature of the ZIF-8 framework enabled efficient DL and EE of CTX. Although the TA- and Cu-modified ZIF-8 DDSs did not enhance the antimicrobial potency of CTX in terms of MIC and MBC values compared to free drug, they preserved bactericidal activity under sustained exposure conditions.

Drug release studies revealed biphasic behavior, which is governed by pH and buffer composition. Phosphate-induced framework destabilization led to accelerated release at pH 7.4, whereas a more gradual, sustained release profile was observed in an acetate buffer at pH 5.0. The prolonged release of CTX for up to 48 h under mildly acidic conditions without abrupt framework collapse suggests that ZIF-8-based maintain controlled local drug availability in microbial infection-relevant environments.

TA and Cu modification primarily contribute to the observed release behavior and carrier characteristics by modulating the coordination environment and surface interactions within the ZIF-8 framework, rather than directly enhancing the antimicrobial potency. Therefore, the ZIF-8 MOF system represents a promising platform for controlled and localized antibiotic delivery, particularly for applications where prolonged exposure at infection sites is desirable, while acknowledging that further *in vivo* investigations are required to assess translational potential and safety.

Ethics

Ethics Committee Approval: There is not required for ethical approval.

Informed Consent: Not required.

Acknowledgements

The authors would like to express their gratitude to Prof. Dr. Mahmut Özacar for granting them access to the laboratory infrastructure and for his invaluable assistance throughout this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.P.T., M.Ö., D.D.Ç., Concept: M.Ö., D.D.Ç., Design: M.Ö., D.D.Ç., Data Collection or Processing: Y.P.T., M.Ö., D.D.Ç., Analysis or Interpretation: Y.P.T., D.D.Ç., Literature Search: Y.P.T., M.Ö., D.D.Ç., Writing: Y.P.T., D.D.Ç.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. AlQurashi DM, AlQurashi TF, Alam RI, Shaikh S, Tarkistani MAM. Advanced nanoparticles in combating antibiotic resistance: current innovations and future directions. J Nanotheranostics. 2025;6:9.
2. Costa BA, Abuçafy MP, Barbosa TWL, da Silva BL, Fulindi RB, Isquibola G, da Costa PI, Chiavacci LA. ZnO@ZIF-8 nanoparticles as nanocarrier of ciprofloxacin for antimicrobial activity. Pharmaceutics. 2023;15:259.
3. Endale H, Mathewos M, Abdeta D. Potential causes of spread of antimicrobial resistance and preventive measures in One Health perspective: a review. Infect Drug Resist. 2023;16:7515-7545.
4. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: a narrative review. Pharmaceuticals (Basel). 2023;16:1615.
5. Al Hagbani T, Rizvi SMD, Hussain T, Mehmood K, Rafi Z, Moin A, Abu Lila AS, Alshammari F, Khafagy ES, Rahamathulla M, Abdallah MH. Cefotaxime mediated synthesis of gold nanoparticles: characterization and antibacterial activity. Polymers (Basel). 2022;14:771.
6. Mihaiescu DE, Istrati D, Morosan A, Stanca M, Purcareanu B, Cristescu R, Vasile BS, Trusca RD. Low release study of cefotaxime by functionalized mesoporous silica nanomaterials. Gels. 2022;8:711.
7. Johnson JK, Laughon MM. Antimicrobial agent dosing in infants. Clin Ther. 2016;38:1948-1960.
8. Javaid S, Ahmad NM, Mahmood A, Nasir H, Iqbal M, Ahmad N, Irshad S. Cefotaxime loaded polycaprolactone based polymeric nanoparticles with antifouling properties for *in-vitro* drug release applications. Polymers (Basel). 2021;13:2180.
9. Mir RA, Weppelmann TA, Teng L, Kirpich A, Elzo MA, Driver JD, Jeong KC. Colonization dynamics of cefotaxime resistant bacteria in beef cattle raised without cephalosporin antibiotics. Front Microbiol. 2018;9:500.
10. Mir RA, Weppelmann TA, Johnson JA, Archer D, Morris JG, Jeong KC. Identification and characterization of cefotaxime resistant bacteria in beef cattle. PLoS One. 2016;11:e0163279.
11. Parvin N, Joo SW, Mandal TK. Nanomaterial-based strategies to combat antibiotic resistance: mechanisms and applications. Antibiotics (Basel). 2025;14:207.
12. Hameed S, Sharif S, Ovais M, Xiong H. Emerging trends and future challenges of advanced 2D nanomaterials for combating bacterial resistance. Bioact Mater. 2024;38:225-257.
13. Aflakian F, Mirzavi F, Aiyelabegan HT, Soleimani A, Navashenaq JG, Karimi-Sani I, Zomorodi AR, Vakili-Ghartavol R. Nanoparticles-based therapeutics for the management of bacterial infections: a special emphasis on FDA approved products and clinical trials. Eur J Pharm Sci. 2023;188:106515.
14. Mendez-Pfeiffer P, Monrreal MGB, Mendez-Encinas MA, Valencia D, Ortiz B, González-Davis O, Cadena-Nava RD. Nanoparticles in antibacterial therapy: a systematic review of enhanced efficacy against intracellular bacteria. ACS Omega. 2025;10:17070-17086.
15. Ohsaki S, Satsuma H, Nakamura H, Watano S. Improvement of solubility of sparingly water-soluble drug triggered by metal-organic framework. J Drug Deliv Sci Technol. 2021;63:102490.
16. Özsoy M, Pirincci Tok Y, Guney Eskiler G, Tok F, Karakus S, Ozsoy Y, Özacar M. The lenalidomide derivative loaded and quercetin modified MIL-100 based novel drug delivery system for breast cancer treatment. J Drug Deliv Sci Technol. 2025;108:106923.
17. Arunkumar T, Castelino E, Lakshmi T, Mulky L, Selvanathan SP, Tahir M. Metal-organic frameworks in antibacterial disinfection: a review. ChemBioEng Rev. 2024;11:e202400006.
18. Yan L, Gopal A, Kashif S, Hazelton P, Lan M, Zhang W, Chen X. Metal organic frameworks for antibacterial applications. Chem Eng J. 2022;435:134975.
19. Wang Y, Tang Y, Guo L, Yang X, Wu S, Yue Y, Xu C. Recent advances in zeolitic imidazolate frameworks as drug delivery systems for cancer therapy. Asian J Pharm Sci. 2025;20:101017.
20. Altun S, Çakıroğlu B, Özacar M, Özacar M. A facile and effective immobilization of glucose oxidase on tannic acid modified $CoFe_2O_4$ magnetic nanoparticles. Colloids Surf B Biointerfaces. 2015;136:963-970.
21. Chevallier P, Wiggers HJ, Copes F, Zorzi Bueno C, Mantovani D. Prolonged antibacterial activity in tannic acid-iron complexed chitosan films for medical device applications. Nanomaterials (Basel). 2023;13:484.
22. Bisht N, Dwivedi N, Kumar P, Venkatesh M, Yadav AK, Mishra D, Solanki P, Verma NK, Lakshminarayanan R, Ramakrishna S, Mondal DP, Srivastava AK, Dhand C. Recent advances in copper and copper-derived materials for antimicrobial resistance and infection control. Curr Opin Biomed Eng. 2022;24:100408.
23. Yu J, Huang X, Ren F, Cao H, Yuan M, Ye T, Xu F. Application of antimicrobial properties of copper. Appl Organomet Chem. 2024;38:e7506.
24. Ozsoy M, Atiroglu V, Guney Eskiler G, Atiroglu A, Arabaci G, Ozacar M. Activities of gallic acid and Fe doped, and glucose oxidase or gold modified ZIF-8 based drug delivery systems in triple negative breast cancer. J Drug Deliv Sci Technol. 2023;87:104878.
25. Resen AK, Atiroğlu A, Atiroğlu V, Guney Eskiler G, Aziz İH, Kaleli S, Özacar M. Effectiveness of 5-fluorouracil and gemcitabine hydrochloride loaded iron-based chitosan-coated MIL-100 composite as an advanced, biocompatible, pH-sensitive and smart drug delivery system on breast cancer therapy. Int J Biol Macromol. 2022;198:175-186.
26. Trucillo P. Drug carriers: a review on the most used mathematical models for drug release. Processes. 2022;10:1094.
27. Pirincci Tok Y, Mesut B, Güngör S, Sarıkaya AO, Aldeniz EE, Dude U, Özsoy Y. Systematic screening study for the selection of proper stabilizers to produce physically stable canagliflozin nanosuspension by wet milling method. Bioengineering (Basel). 2023;10:927.

28. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Wayne (PA): CLSI; 2006.

29. National Committee for Clinical Laboratory Standards. Methods for determining bactericidal activity of antimicrobial agents: approved guideline M26-A. Wayne (PA): NCCLS; 1999.

30. Kaur H, Mohanta GC, Gupta V, Kukkar D, Tyagi S. Synthesis and characterization of ZIF-8 nanoparticles for controlled release of 6-mercaptopurine drug. J Drug Deliv Sci Technol. 2017;41:106-112.

31. El-Damhougy TK, Ahmed ASI, Gaber GA, Mazied NA, Bassioni G. Radiation synthesis for a highly sensitive colorimetric hydrogel sensor-based p(AAc/AMPS)-TA for metal ion detection. Results Mater. 2021;9:100169.

32. Latrach Z, Moumen E, Kounbach S, El Hankari S. Mixed-ligand strategy for the creation of hierarchical porous ZIF-8 for enhanced adsorption of copper ions. ACS Omega. 2022;7:15862-15869.

33. Khafaga DSR, El-Morsy MT, Faried H, Diab AH, Shehab S, Saleh AM, Ali GAM. Metal-organic frameworks in drug delivery: engineering versatile platforms for therapeutic applications. RSC Adv. 2024;14:30201-30229.

34. Yang S, Lü F, Wang L, Liu S, Wu Z, Cheng Y, Liu F. pH-responsive metal-organic framework for targeted delivery of fungicide, release behavior, and sustainable plant protection. Molecules. 2024;29:5330.

35. Velásquez-Hernández MJ, Ricco R, Carraro F, Limpoco FT, Linares-Moreau M, Leitner E, Wiltsche H, Rattenberger J, Schröttner H, Frühwirt P, Stadler EM, Gescheidt G, Amenitsch H, Doonan CJ, Falcaro P. Degradation of ZIF-8 in phosphate buffered saline media. CrystEngComm. 2019;21:4538-4544.

36. Li D, Li J, Wang S, Wang Q, Teng W. Dually crosslinked copper-poly(tannic acid) nanoparticles with microenvironment-responsiveness for infected wound treatment. Adv Healthc Mater. 2023;12:e2203063.

37. Xiong MH, Bao Y, Yang XZ, Zhu YH, Wang J. Delivery of antibiotics with polymeric particles. Adv Drug Deliv Rev. 2014;78:63-76.

38. Razei A, Cheraghali AM, Saadati M, Fasihi Ramandi M, Panahi Y, Hajizadeh A, Siadat SD, Behrouzi A. Gentamicin-loaded chitosan nanoparticles improve its therapeutic effects on Brucella-infected J774A.1 murine cells. Galen Med J. 2019;8:e1296.

39. Ahmed SA, Hasan N, Bagchi D, Altass HM, Morad M, Althagafi II, Hameed AM, Sayqal A, Khder AERS, Asghar BH, Katouah HA, Pal SK. Nano-MOFs as targeted drug delivery agents to combat antibiotic-resistant bacterial infections. R Soc Open Sci. 2020;7:200959.

40. Nabipour H, Aliakbari F, Volkening K, Strong MJ, Rohani S. Novel metal-organic framework coated with chitosan-κ-carrageenan as a platform for curcumin delivery to cancer cells. Int J Biol Macromol. 2025;301:140027.

41. Stiepel RT, Pena EP, Ehrenzeller SA, Gallovic MD, Lifshits LM, Genito CJ, Bachelder EM, Ainslie KM. A predictive mechanistic model of drug release from surface eroding polymeric nanoparticles. J Control Release. 2022;351:883-895.

42. Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev. 2013;65:1803-1815.

43. Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol. 2013;11:371-384.

44. Levison ME, Levison JH. Pharmacokinetics and pharmacodynamics of antibacterial agents. Infect Dis Clin North Am. 2009;23:791-815.

Turk J Pharm Sci 2026;23(1):41-49
DOI: 10.4274/tjps.galenos.2026.09090

ORIGINAL ARTICLE

Missed Opportunities in Osteoporosis Prevention and Screening in Postmenopausal Women: Risk Profiles and Awareness

Fatma ÖZDOĞAN[1], Burcu KELLECİ ÇAKIR[1]*, Şerife Gül ÖZ[2], Aygin BAYRAKTAR-EKİNCİOĞLU[1]

[1]Hacettepe University Faculty of Pharmacy, Department of Clinical Pharmacy, Ankara, Türkiye
[2]Hacettepe University Faculty of Pharmacy, Department of Internal Medicine, Ankara, Türkiye

ABSTRACT

Objectives: Osteoporosis (OP) is a major public health issue among postmenopausal women. Despite its high prevalence, awareness and screening practices remain insufficient. This study aims to evaluate OP-related risk factors and awareness levels among postmenopausal women.

Materials and Methods: This prospective study was conducted between August 2023 and April 2024 among patients admitted to the general internal medicine outpatient clinic of a tertiary care hospital. Participants were categorized into two groups: patients with OP and patients without OP (non-OP). OP awareness was assessed using the OP awareness scale.

Results: The study included 206 participants: 101 in the OP group and 105 in the non-OP group. Evaluation of risk factors (n = 20) showed no significant difference (SD) in the mean number of risk factors between the groups [OP: 7.29 (1.77), non-OP: 6.71 (1.94), $p > 0.05$]. Both groups demonstrated inadequate physical activity and dietary habits. The OP group demonstrated a higher prevalence of glucocorticoid use, while the non-OP group exhibited a higher prevalence of vitamin D deficiency ($p < 0.01$) and type 2 diabetes ($p = 0.02$). About 35% of the non-OP group have not undergone bone-mineral-density measurements within the last 2 years, and 11% of these individuals were identified as high risk for major osteoporotic fracture within a 10-year timeframe. There was no SD in OP awareness between the groups [mean (SD) scores: OP 61.18 (16.02) and non-OP 61.32 (14.39); $p = 0.44$]. Conversely, risk of OP was positively correlated with patient age ($p = 0.004$), lower awareness score in the protective behavior subdomain ($p = 0.02$), and lower body mass index ($p < 0.001$).

Conclusion: OP prevention and its management require patient-specific strategies that include educational interventions to increase awareness and routine screening for risk factors among postmenopausal women. Pharmacists are well-positioned to identify patients' health-related and educational needs.

Keywords: Osteoporosis, awareness, risk factors, postmenopausal, clinical pharmacist

INTRODUCTION

Osteoporosis (OP) is a silent yet pervasive condition that disproportionately affects women, particularly in the postmenopausal period, due to the decline in circulating estrogen levels, a hormone crucial for bone density maintenance.[1] Recognized as a pressing public health concern, OP leads to the gradual deterioration of bone health and a heightened risk of fractures, often remaining undetected until such fractures occur.[2,3] The risk is especially pronounced among postmenopausal women, whose vulnerability is linked to hormonal changes following menopause.[4] While primary OP is often associated with aging and menopause and is therefore commonly observed in postmenopausal women and older adults of all genders, secondary OP transcends age and gender categories. Characterized by low bone mineral density (BMD), secondary OP increases the risk of fragility fractures

***Correspondence:** burcukelleci@hacettepe.edu.tr, **ORCID-ID:** orcid.org/0000-0003-2547-8919
Received: 07.02.2026, **Accepted:** 20.04.2026 **Epub:** 15.05.2026 **Publication Date:** 13.07.2026

Cite this article as: Özdoğan F, Kelleci Çakır B, Öz ŞG, Bayraktar-Ekincioğlu A. Missed opportunities in osteoporosis prevention and screening in postmenopausal women: risk profiles and awareness. Turk J Pharm Sci. 2026;23(1):41-49

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

due to various underlying diseases or medication use.[5,6] The differentiation between primary and secondary OP not only reflects biomedical classification but also prompts critical analysis of the ways in which gendered expectations and medical discourse impact bone health and aging experiences. As have been highlighted, a biomedical perspective on women's health in later life frequently ignores the sociocultural aspects of aging femininity and physical vulnerability.[7] Therefore, an approach to understanding OP must take into account both biological mechanisms and the gendered narratives surrounding aging, fragility, and the female body.

Despite its high prevalence, OP remains underrecognized, particularly among postmenopausal women. Many continue to underestimate the significance of preventive measures, remaining unaware of the potential for fractures and other complications associated with progressive bone loss.[8] While BMD measurement is a key clinical tool in assessing fracture risk,[9] recent guidelines emphasize that clinical decisions should not rely solely on BMD; rather, a comprehensive evaluation of fracture risk factors is essential.[10,11] This includes attention to comorbidities, medication use, and lifestyle-related contributors, factors often shaped by socioeconomic status, gendered caregiving roles, and limited access to health education.[12]

Enhancing early awareness, well before a fracture occurs, is thus critical in managing OP effectively.[12,13] Importantly, knowledge-building interventions must go beyond generic public health messaging to account for how gender, age, and structural inequalities overlap in shaping women's access to information and care. Empowering women with knowledge about OP through culturally and socially sensitive education strategies may enable more proactive approaches to bone health, such as weight-bearing physical activity and adequate intake of calcium and vitamin D.[14]

Against this backdrop, the present study aims to evaluate OP-related risk factors and awareness levels among postmenopausal women attending a general internal medicine outpatient clinic.

MATERIALS AND METHODS

This prospective study was conducted between August 2023 and April 2024, targeting postmenopausal women aged 45–64 years (considered a vulnerable group regarding OP prevention) who attended the general internal medicine outpatient clinic of a tertiary care hospital. Women diagnosed with OP prior to menopause or falling outside the specified age range were excluded. Participants were included after providing written informed consent, and ethical approval was obtained from the Hacettepe University Health Sciences Research Ethics Committee (approval number: 2023/04-35; dated 12.09.2023).

The study population consists of postmenopausal women who were admitted to the general internal medicine outpatient clinic at the Hacettepe University Hospital. Since the study was observational in nature, no additional sample size calculation was performed for any assumption. All patients who met the inclusion criteria during the study period constituted the study sample. The power analysis was performed at the end of the study.

OP risk factors were assessed using a combination of the eleven parameters defined by the World Health Organization-endorsed FRAX tool and nine additional factors identified through a review of recent literature. In line with Fracture Risk Assessment Tool (FRAX) criteria, the study defined 'advanced age' as 55–64 years. The FRAX algorithm was used to estimate the 10-year probability of a major osteoporotic fracture (MOF) in women without a prior diagnosis of OP. When the estimated MOF risk was ≥10% and no BMD test had been performed within the last two years, physicians were informed to facilitate appropriate clinical follow-up.

To evaluate participants' awareness of OP, the validated Turkish version of the OP awareness scale (OAS) was administered.[15,16] The scale comprises 27 items across five subdomains: bone physiology, protective behaviors, risk factors, exercise, and general characteristics of the disease. Scores range from 27 to 108, with higher scores reflecting greater awareness. In addition to the scale, participants responded to 12 questions designed by the research team to assess knowledge of OP-related risks. These questions aimed to capture not only clinical knowledge but also contextual and experiential dimensions of women's understanding of and relationship to their bone health.

Statistical analysis

The data were presented as means and standard deviations or as medians and minimum-maximum values for numerical variables, and as numbers and percentages for categorical variables. The normality assumption was examined by the Shapiro–Wilk test. For group comparisons, a significance test for the difference between two means was performed when parametric test assumptions were met; when they were not, the Mann–Whitney U test was used. The relationship between numerical variables was analyzed using the Pearson correlation test. The significance level was set at $p < 0.05$. All analyses were performed using IBM SPSS v.23.

A post-hoc power analysis was performed using G*Power 3.1 to evaluate the adequacy of the sample size for two separately defined sets of OP risk factors. Among the eleven FRAX-based risk factors, the mean number was 3.73 [significant difference (SD) = 1.06] in the OP group and 2.80 (SD = 1.16) in the non-OP group, yielding a Cohen's d of 0.84. With sample sizes of 101 and 105, respectively, and $\alpha = 0.05$ (two-tailed), the achieved statistical power was $1\text{-}\beta = 1.00$. For the nine literature-based additional risk factors, the mean was 3.56 (SD = 1.35) in the OP group and 2.80 (SD = 1.58) in the non-OP group, corresponding to a Cohen's d of 0.52 and an achieved power of $1\text{-}\beta = 0.96$. Both analyses confirm that the study was sufficiently powered to detect clinically meaningful differences in the burden of OP risk factors between groups.

RESULTS

A total of 206 postmenopausal women were included in the study, of whom 101 were diagnosed with OP and 105 were not. The mean age was slightly higher in women with OP [58.78 (SD: 3.95) years] than in those without OP [57.33 (SD = 4.23)

years], although the difference was not statistically significant (p = 0.26) (Table 1). The prevalence of chronic diseases differed between the groups: hypertension was more common among women with OP (42.6%), while type 2 diabetes was most frequent among those without OP (46.7%).

Patterns of medication use reflect both clinical need and broader gendered dynamics in aging. Vitamin D3 supplements were the most frequently used medications in the OP group (55.4%), followed by cardiovascular medications (47.5%) and thyroid medications (33.7%). In contrast, women without OP were most commonly prescribed cardiovascular drugs (50.5%), antidiabetic drugs (44.8%), and dyslipidemia-related drugs (36.2%). Unsurprisingly, use of vitamin D3 and calcium was significantly higher in the OP group (p < 0.001 and p = 0.02, respectively), while use of antidiabetic medication was more common in the non-OP group (p = 0.003). Of the women diagnosed with OP,

Table 1. Characteristics of the participants.

	With OP (n = 101)	Without OP (n = 105)	*p*
	n (%)		
Age (years) mean (SD)	58.78 (3.95)	57.33 (4.23)	0.26
Age of menopause (years) mean (SD)	46.3 (5.02)	47.28 (4.98)	0.68
Early menopause (<45 years)	25 (24.8)	26 (24.8)	0.9
Age of menarche (years) mean (SD)	13.15 (1.33)	13.06 (1.71)	0.05
Duration of breastfeeding (years) mean (SD)	3.22 (2.29)	3.02 (2.35)	0.91
Duration of OP (months) mean (SD)	40.24 (40.91)	-	N/A
Number of drugs used per day mean (SD)	4.69 (3.32)	4.48 (3.54)	0.60
Body mass index (kg/m²)			
<18.5	2 (2)	0 (0)	<0.001
18.6–24.9	32 (31.7)	12 (11.4)	
25–29.9	36 (35.6)	37 (35.2)	
≥30 (obese)	31 (30.7)	56 (53.3)	
Level of education			
No formal education	2 (2)	8 (7.6)	0.29
Primary school	60 (59.4)	61 (58.1)	
High school	24 (23.8)	21 (20.0)	
University	15 (14.9)	15 (14.3)	
Number of births			
None	8 (7.9)	5 (4.8)	0.53
1–3	73 (72.3)	82 (78.1)	
>3	20 (19.8)	18 (17.1)	
Number of comorbid diseases, mean (SD)	3.47 (1.50)	2.91 (1.71)	0.31
Comorbidities			
Type II diabetes	33 (32.7)	49 (46.7)	0.04
Hypertension	43 (42.6)	47 (44.8)	0.75
Hypothyroidism	32 (31.7)	28 (26.7)	0.42
Hyperlipidemia	30 (29.7)	40 (38.1)	0.2
Asthma	8 (7.9)	15 (14.2)	0.14
Coronary artery disease	6 (5.9)	9 (8.6)	0.46
Alkaline phosphatase, median (IQR)	70 (28)	77 (26.5)	0.01

SD, standard deviation; IQR, interquartile range; OP, osteoporosis; N/A, not applicable.

57 were receiving pharmacological treatment: of these, 40.5% were prescribed bisphosphonates, 5.9% were prescribed a bisphosphonate-calcium combination, 6.9% were prescribed calcium carbonate alone, 2% were prescribed denosumab, and 1% were prescribed a denosumab-calcium combination. All women who were taking at least one medication received counselling by a clinical pharmacist, reflecting the study's emphasis on comprehensive pharmaceutical care.

Biochemical parameters (albumin, calcium, phosphorus, thyroid stimulating hormone, glomerular filtration rate, and creatinine) did not differ significantly between the two groups ($p > 0.05$). However, median alkaline phosphatase levels were significantly higher in women without OP ($p = 0.01$), a finding that warrants further investigation. Among women without OP, 65% (n = 68) had undergone BMD testing within the past two years (Table 2).[17]

Evaluation of risk factors showed that the OP group had averages of 3.73 (SD = 1.06) out of 11 FRAX risk factors and 3.56 (SD = 1.35) out of 9 additional literature-based risk factors. In the non-OP group, the averages were 2.8 (SD = 1.16) and 3.9 (SD = 1.58), respectively. When considering all 20 risk factors, the OP group had a mean of 7.29 (SD = 1.77), and the non-OP group had a mean of 6.71 (SD = 1.94); this difference was not statistically significant ($p > 0.05$). Glucocorticoid use was notably higher among women with OP, while vitamin D deficiency ($p < 0.01$) and type 2 diabetes ($p = 0.02$) were more prevalent among women without OP (Table 3).

Interestingly, post-hoc power analysis (two-tailed independent samples t-test, $\alpha = 0.05$, observed effect size d = 0.009) indicated that the statistical power for detecting this difference was negligible ($1-\beta < 0.06$), and overall OAS did not differ significantly between the groups (Table 4). This near-zero effect size indicates that the lack of a statistically SD (SD) in OP awareness between groups constitutes a substantive finding, suggesting that an OP diagnosis does not meaningfully improve awareness. However, when participants were stratified by educational background, participants with at least a high school education scored significantly higher on the OAS ($p < 0.01$), highlighting the role of educational inequality in shaping health literacy among women.

A weak but statistically significant negative correlation between the total number of OP risk factors and OAS scores (Table 5) suggests that higher risk does not necessarily translate into higher awareness. Logistic regression analysis revealed three variables associated with increased risk of OP: increasing age [odds ratio (OR): 1.17, 95% confidence interval (CI): 1.053–1.306, $p = 0.004$], lower scores in the "protective behaviors" subdomain of the OAS (OR: 0.85, 95% CI: 0.729–0.981, $p = 0.02$), and lower body mass index (BMI) values (OR: 0.81, 95% CI: 0.729–0.891, $p < 0.001$).

Regarding the total patient population (n = 206), it is evident that patients are not cognizant that certain medications (e.g., proton pump inhibitors, antidepressants, antiepileptics, hormonal agents, and drugs for thyroid diseases), certain clinical conditions (e.g., gastrointestinal system disorders, rheumatological and endocrine disorders), and reproductive and hormonal factors in women (e.g., vitamin D deficiency, duration of lactation >1 year, >3 pregnancies, and late menarche) can increase the risk of developing OP (Figure 1).

A significant discrepancy was identified when comparing patients' (group of OP versus non-OP) responses to questions assessing their knowledge of OP risk factors. The only SD was found in responses to the question, "Some stomach-protecting medications may increase the risk of OP" ($p = 0.03$). No SD was found in responses to the other questions. In the Kruskal-Wallis analysis conducted to ascertain the relationship between the number of risk factors present in patients and their responses to questions related to risk factors, a SD was identified between the responses to the question "Vitamin D deficiency can increase the risk of OP" and the number of risk factors identified in patients ($p < 0.001$). Patients who responded "I don't know" to this question had a significantly higher number of identified risk factors than those who responded "I know" ($p < 0.001$).

DISCUSSION

Despite the high prevalence of OP among postmenopausal women, this study highlights a persistent gap in both the comprehensive assessment of OP risk factors and the awareness of the condition among affected populations. It is noteworthy that the comparable number of risk factors

Table 2. 10-year probability of fracture (%) in patients without osteoporosis (n = 105).

	BMD measurements performed within the last 2 years	
	Yes (n = 68)	No (n = 37)
	Median (minimum-maximum)	
MOF	4.3 (2.5–12.1)	3.9 (2.5–15)
Hip fracture	0.55 (0–6.5)	0.5 (0.1–1.9)
Patients at risk according to the MOF-FRAX score;[a]	n (%)	
Low-risk, <10% (to be monitored by BMD)	67 (98.5)	33 (89.2)
High-risk, ≥10% (to be treated)	1 (1.5)	4 (10.8)

[a]The cut-off value was accepted as 10% from Kanis et al.[17] 2008. BMD, bone mineral density; MOF, major osteoporotic fracture.

and similar levels of awareness between women with and without OP indicate a potential communication failure between healthcare providers and patients. This emphasises a need for an established pathway that can be facilitated by a pharmacist to ensure ongoing patient care between primary and secondary care.[18]

This communication gap likely reflects intersecting barriers related to patients' health literacy, physicians' screening practices, and broader structural factors within healthcare systems. Existing literature suggests that overcoming these barriers demands proactive risk assessment, adherence to guideline-recommended BMD screenings, and importantly, multidisciplinary interventions, including pharmacist-led education for both clinicians and patients.[19-21] The finding that over a third of women without OP either lacked recent BMD measurements or had no BMD measurements underscores inequities in access to healthcare and highlights the need for community-based awareness campaigns tailored to the sociocultural realities of women in this demographic.

Table 3. Presence of OP risk factors among the participants.

	OP group (n = 101)	Non-OP group (n = 105)	
Risk factors	**n (%)**		***p***
Included in the FRAX			
Advancing age (55–64 years)	85 (84.2)	78 (74.2)	0.05
Body mass index (<20 kg/m^2)	5 (4.9)	0	-
Gender (female)	101 (100)	105 (100)	-
Current smoker	19 (18.8)	21 (20)	0.86
Glucocorticoid use	18 (17.8)	7 (6.7)	0.01
Previous fracture	12 (11.9)	0	-
Family history of hip fracture	10 (9.9)	13 (12.4)	0.57
Rheumatoid arthritis	7 (6.9)	4 (3.8)	0.31
Secondary OP [early menopause (<45 years) and chronic liver disease]	27 (26.7)	27 (25.7)	0.87
Alcohol use	0	0	-
BMD	95 (93.1)	40 (38.1)	<0.01
Additional/other risk factors			
Inadequate physical activity (less than 3 x 30 min/week)	58 (57.4)	57 (54.2)	0.65
Lack of healthy eating habits (less than 2 portion of milk or diary products/day)	55 (54.4)	68 (64.7)	0.13
Vitamin D deficiency (<20 ng/mL)	11 (10.9)	34 (32.4)	<0.01
Comorbid diseases	48 (47.5)	58 (55.2)	0.26
Type 2 diabetes	32 (31.7)	49 (46.7)	0.02
SLE/AS	2	6	0.16
IBS/Crohn's/Celiac	1	2	0.58
CKD	1	2	0.58
COPD	-	2	-
Other medicines	53 (52.5)	55 (52.4)	0.55
Proton pump inhibitor	25 (24.8)	22 (21)	0.51
Levothyroxine	11 (10.9)	15 (14.3)	0.46
Selective serotonin reuptake inhibitor	32 (31.7)	31 (29.5)	0.73
Late (≥15 years) menarche	14 (13.8)	19 (18.1)	0.4
Number of pregnancies (>3)	20 (19.8)	19 (18.1)	0.75
Long term (>1 year) lactation	76 (75.2)	75 (71.4)	0.53
Bilateral oophorectomy/hysterectomy	25 (24.8)	25 (23.8)	0.87

BMD, bone mineral density; OP, osteoporosis; COPD, chronic obstructive pulmonary disease; CK, creatine kinase.

In this study, we found that 35.2% of women without OP had not undergone a BMD measurement within the past two years or had never been screened during their lifetime. Previous research similarly reports OP screening rates ranging between 20% and 44% among women.[22,23] While clinical guidelines recommend regular BMD assessments for postmenopausal women aged 65 and over, as well as for younger women presenting risk factors,[24,25] significant disparities in access to healthcare services, limited awareness of OP risk factors, and variations in physicians' screening practices contribute to inconsistent screening uptake. Therefore, this study identified and characterized the OP risk profiles and awareness gaps in this pre-guideline window where opportunities for early prevention are frequently missed. These disparities often overlap with broader structural inequalities related to gender, socioeconomic status, and healthcare accessibility. Hence, the findings underscore the need for community-based awareness initiatives that are culturally sensitive and tailored to the specific needs and barriers of diverse groups of women. Additionally, a comprehensive evaluation of all OP risk factors in the population is essential to ensure early detection and equitable preventive care.

Menopause heralds a complex metabolic transition, increasing risks not only for OP but also for comorbid conditions such as diabetes and cardiovascular disease, conditions that disproportionately affect women.[26] The differential prevalence of comorbidities and associated medication use observed between the groups further underscores how medical risk factors are shaped by lifestyle, social roles, and healthcare experiences.

Bone loss accelerates during the menopausal transition, with an estimated annual decline of 2% that begins 1 to 3 years prior to menopause and continues for 5 to 10 years. This process culminates in a significant reduction of BMD by about 10–12% in the spine and hip regions.[24] The prevalence of comorbidities such as diabetes and hypertension in both study groups suggests a complex interplay between metabolic health and bone integrity during midlife. While obesity is often conceptualized as a risk factor for various metabolic conditions, the present study's finding that women with OP tend to have lower BMIs aligns with emerging evidence on the protective role of adipose-derived estrogen in bone health.[27,28] Conversely, vitamin D deficiency and type 2 diabetes, more prevalent in the non-osteoporotic group, highlight the complex and sometimes paradoxical nature of risk profiles in postmenopausal women.

OAS may help identify patients with insufficient knowledge who could benefit from targeted educational interventions. In clinical practice, these scores may support healthcare professionals in prioritizing patient counseling and improving OP prevention strategies. The wide range in OP awareness

Table 4. OAS scores.

	OP group	Non-OP group	*p*
Subdomains	Mean (±SD)		
Bone physiology	13.50 (4.33)	13.7 (4.26)	0.52
Protective behaviors	15.66 (4.6)	15.7 (4.28)	0.48
Risk factors	8.86 (3.44)	8.9 (3.28)	0.65
Exercise	8.46 (3.71)	8.53 (3.38)	0.18
Characteristics of OP	14.72 (3.49)	14.34 (3.36)	0.77
OAS total score	61.18 (16.02)	61.32 (14.39)	0.44

OAS, Osteoporosis Awareness Scale; SD, standard deviation; OP, osteoporosis.

Table 5. The relationship between the OAS score and the number of risk factors.

Bone physiology		OAS subgroups					OAS total score
		Protective behaviors	Risk factors	Exercise	Characteristics OP		
Number of FRAX risks (n = 11)	r	0.030	0.072	0.052	0.076	0.105	0.076
	p	0.665	0.304	0.458	0.276	0.132	0.276
Number of other risks (n = 9)	r	-0.272	-0.292	-0.195	-0.238	-0.327	-0.329
	p	<0.001	<0.001	0.005	<0.001	<0.001	<0.001
Total number of risks (n = 20)	r	-0.195	-0.184	-0.120	-0.138	-0.190	-0.21
	p	0.002	0.005	0.08	0.048	0.006	0.002

OAS, Osteoporosis Awareness Scale; OP, osteoporosis.

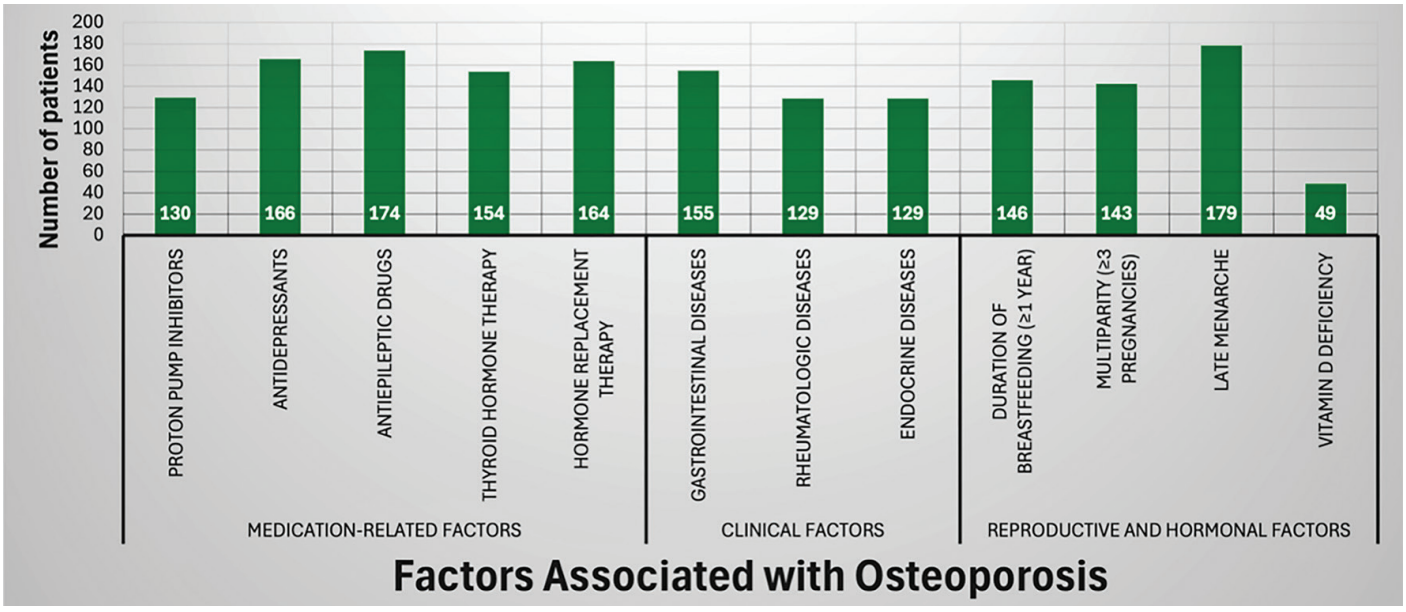


Figure 1. Patients indicated as "I don't know at all" about OP risk factors.
OP, osteoporosis.

around the world, from as low as 8% to nearly 70% in different countries,[18] highlights the need for educational interventions that are culturally sensitive and tailored to specific contexts. While numerous studies have employed various questionnaires and scales to assess OP awareness and knowledge,[12,23,29-32] there remains a paucity of research specifically comparing the levels of OP-related knowledge between postmenopausal women with and without the condition. This study emphasizes that women's knowledge of OP and its associated risks is linked to women's educational level, which is particularly important at earlier ages to increase awareness and promote self-care.

Study results confirm the impact of aging as a non-modifiable but critical risk factor, while emphasizing modifiable factors such as awareness of protective behaviors and BMI. This highlights the potential for targeted cognitive-behavioral and lifestyle interventions to mitigate OP risks.[19,33,34] Consistent with previous research, higher OP knowledge or awareness correlates with engagement in protective behaviors, including calcium and vitamin D supplementation, regular exercise, and sufficient consumption of dairy products.[19,36,37] Nutrition plays a critical protective role; conversely, low BMI contributes to muscle weakness, reduced BMD, and decreased estrogen production from adipose tissue, all of which increase the risk of OP.[27,28,37-40]

Study limitations

The study has inevitable limitations, such as being conducted at a single center and having a relatively small sample size. However, this study has focused on a comprehensive assessment of risk factors among postmenopausal women, including medical, pharmacological, and lifestyle factors.

CONCLUSION

The postmenopausal period is characterized by accelerated bone loss, which is further exacerbated by risk factors deeply linked with social, behavioral, and structural determinants of health. This study highlights a critical need for comprehensive assessment of both biological risk factors and OP awareness among postmenopausal women. Effective management of bone health should include not only clinical measures but also culturally modified education and equitable access to screening and preventive services.

Fostering greater awareness and empowering women with knowledge tailored to their experiences and socio-cultural contexts can contribute to reduced fracture risk and improved quality of life.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Hacettepe University Health Sciences Research Ethics Committee (approval number: 2023/04-35; dated 12.09.2023).

Informed Consent: Participants were included after providing written informed consent.

Acknowledgements

We are grateful to the healthcare workers in the Internal Medicine Outpatient Clinic at Hacettepe University for their contributions.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ş.G.Ö., Concept: F.Ö., B.K.Ç., Ş.G.Ö., A.B.-E., Design: Ş.G.Ö., A.B.-E., Data Collection or Processing: F.Ö., Analysis or Interpretation: B.K.Ç., Literature Search: F.Ö., B.K.Ç., Writing: F.Ö., B.K.Ç., Ş.G.Ö., A.B.-E.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Ang JL, Hodara E, Sriprasert I, Shoupe D, Stanczyk FZ. Estrogen deficiency in the menopause and the role of hormone therapy: integrating the findings of basic science research with clinical trials. Menopause. 2024;31:926-939.
2. Johnston CB, Dagar M. Osteoporosis in older adults. Med Clin North Am. 2020;104:873-884.
3. Ebeling PR, Nguyen HH, Aleksova J, Vincent AJ, Wong P, Milat F. Secondary osteoporosis. Endocrine Rev. 2021;43:240-313.
4. Paik J, Scott LJ. Romosozumab: a review in postmenopausal osteoporosis. Drugs Aging. 2020;37:845-855.
5. Emkey GR, Epstein S. Secondary osteoporosis: pathophysiology & diagnosis. Best Pract Res Clin Endocrinol Metab. 2014;28:911-935.
6. Kelman A, Lane NE. The management of secondary osteoporosis. Best Pract Res Clin Rheumatol. 2005;19:1021-1037.
7. McKinney JL, Clinton SC, Keyser LE. Women's health across the lifespan: a sex- and gender-focused perspective. Phys Ther. 2024;104.
8. Gerend MA, Erchull MJ, Aiken LS, Maner JK. Reasons and risk: factors underlying women's perceptions of susceptibility to osteoporosis. Maturitas. 2006;55:227-237.
9. Bahat G, Catikkas NM, Yavuz DG, Borman P, Guzel R, Reginster JY. The current situation in the approach to osteoporosis in older adults in Turkey: areas in need of improvement with a model for other populations. Arch Osteoporos. 2021;16:179.
10. Türkiye Endokrinoloji ve Metabolizma Derneği. Osteoporoz ve metabolik kemik hastalıkları tanı ve tedavi kılavuzu. 2022.
11. Bernabei R, Martone AM, Ortolani E, Landi F, Marzetti E. Screening, diagnosis and treatment of osteoporosis: a brief review. Clin Cases Miner Bone Metab. 2014;11:201-207.
12. Gopinathan NR, Sen RK, Behera P, Aggarwal S, Khandelwal N, Sen M. Awareness of osteoporosis in postmenopausal Indian women: an evaluation of osteoporosis health belief scale. J Midlife Health. 2016;7:180-184.
13. Tardi P, Szilagyi B, Makai A, Gyuro M, Acs P, Jaromi M, Molics B, Hock M. The development of a reliable and valid instrument to measure the osteoporosis-related knowledge: validation of the Hungarian version of Osteoporosis Knowledge Assessment Tool (OKAT). BMC Public Health. 2021;21:1515.
14. Benton MJ. Osteoporosis: recommendations for resistance exercise and supplementation with calcium and vitamin D to promote bone health. J Commun Health Nurs. 2006;23:201-211.
15. Choi ES, Kim JH, Chung MY, Hwang KH. Development of an osteoporosis awareness scale for women. J Korean Acad Nurs. 2008;38:813-821.
16. Ocak Aktürk S. Osteoporoz farkındalık ölçeğinin Türkçe formunun geçerlilik ve güvenilirlik çalışması [Doktora Tezi]. İzmir: Ege Üniversitesi; 2019.
17. Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A, National Osteoporosis Guideline G. Case finding for the management of osteoporosis with FRAX—assessment and intervention thresholds for the UK. Osteoporos Int. 2008;19:1395-1408.
18. Tan HC, Seng JJB, Low LL. Osteoporosis awareness among patients in Singapore (OASIS) - a community hospital perspective. Arch Osteoporos. 2021;16:151.
19. Saltık H, Öztürk F, Emiroğlu C, Hekimoğlu B, Aypak C. Knowledge, attitude, and behavior levels of postmenopausal women about osteoporosis. J Bone Metab. 2023;30:347-354.
20. Solomon DH, Polinski JM, Stedman M, Truppo C, Breiner L, Egan C, Jan S, Patel M, Weiss TW, Chen YT, Brookhart MA. Improving care of patients at-risk for osteoporosis: a randomized controlled trial. J Gen Intern Med. 2007;22:362-367.
21. Tso LS, Loi D, Mosley DG, Yi D, Stockl KM, Lew HC, Solow BK. Evaluation of a nationwide pharmacist-led phone outreach program to improve osteoporosis management in older women with recently sustained fractures. J Manag Care Spec Pharm. 2015;21:803-810.
22. Amarnath AL, Franks P, Robbins JA, Xing G, Fenton JJ. Underuse and overuse of osteoporosis screening in a regional health system: a retrospective cohort study. Gen Intern Med. 2015;30:1733-1740.
23. Lewiecki EM, Leader D, Weiss R, Williams SA. Challenges in osteoporosis awareness and management: results from a survey of US postmenopausal women. J Drug Assess. 2019;8:25-31.
24. Management of osteoporosis in postmenopausal women: the 2021 position statement of The North American Menopause Society. Menopause. 2021;28:973-997.
25. Force UPST. Screening for osteoporosis to prevent fractures: US Preventive Services Task Force recommendation statement. JAMA. 2018;319:2521-2531.
26. Thaung Zaw JJ, Howe PRC, Wong RHX. Postmenopausal health interventions: time to move on from the Women's Health Initiative? Ageing Res Rev. 2018;48:79-86.
27. Patel DB, Nosal BM, Mofrad MD, Chun OK. Identification of the risk factors associated with low bone density in peri- and early postmenopausal women. Dietetics. 2024;3:75-86.
28. Liu Y, Liu Y, Huang Y, Le S, Jiang H, Ruan B, Ao X, Shi X, Fu X, Wang S. The effect of overweight or obesity on osteoporosis: a systematic review and meta-analysis. Clin Nutr. 2023;42:2457-2467.
29. Kale A, Khandelwal N, Sirohi B, Shaki O, Rai S. Knowledge, attitudes, practices, and awareness levels among Indian postmenopausal women about osteoporosis and its relationship with sociodemographic factors: a cross-sectional study from northern India. Cureus. 2024;16:e59606.
30. Kim Y, Kim JH, Cho DS. [Gender difference in osteoporosis prevalence, awareness and treatment: based on the Korea national health and nutrition examination survey 2008~2011]. J Korean Acad Nurs. 2015;45:293-305.
31. Rundasa DT, Ayisa AA, Mekonen EG. Knowledge, health belief, and associated factors towards the prevention of osteoporosis among post-menopausal women in Metu Town, southwest Ethiopia: a community-based cross-sectional study. Int J Orthop Trauma Nurs. 2022;45:100905.
32. Tabor E, Grodzki A, Pluskiewicz W. Higher education and better knowledge of osteoporosis improve bone health in Polish postmenopausal women. Endokrynol Pol. 2022;73:831-836.
33. Alexandraki KI, Syriou V, Ziakas PD, Apostolopoulos NV, Alexandrakis AI, Piperi C, Kavoulaki E, Myriokefalitakis I, Korres G, Diamanti-Kandarakis E. The knowledge of osteoporosis risk factors in a Greek female population. Maturitas. 2008;59:38-45.
34. Asaoka D, Nagahara A, Shimada Y, Matsumoto K, Ueyama H, Matsumoto K, Nakagawa Y, Takeda T, Tanaka I, Sasaki H, Osada T, Hojo M, Watanabe S. Risk factors for osteoporosis in Japan: is it associated with Helicobacter pylori? Ther Clin Risk Manag. 2015;11:381-391.

35. El Hage C, Hallit S, Akel M, Dagher E. Osteoporosis awareness and health beliefs among Lebanese women aged 40 years and above. Osteoporos Int. 2019;30:771-786.

36. Kutsal YG, Atalay A, Arslan Ş, Başaran A, Cantürk F, Cindaş A, Eryavuz M, İrdesel J, Karadavut Kİ, Kirazlı Y, Sindel D, Şenel K, Güler-Uysal F, Yıldırım K. Awareness of osteoporotic patients. Osteoporos Int. 2005;16:128-133.

37. Xiang BY, Huang W, Zhou GQ, Hu N, Chen H, Chen C. Body mass index and the risk of low bone mass-related fractures in women compared with men: a PRISMA-compliant meta-analysis of prospective cohort studies. Medicine (Baltimore). 2017;96:e5290.

38. Chiu CT, Lee JI, Lu CC, Huang SP, Chen SC, Geng JH. The association between body mass index and osteoporosis in a Taiwanese population: a cross-sectional and longitudinal study. Sci Rep. 2024;14:8509.

39. Cui R, Zhou L, Li Z, Li Q, Qi Z, Zhang J. Assessment risk of osteoporosis in Chinese people: relationship among body mass index, serum lipid profiles, blood glucose, and bone mineral density. Clin Interv Aging. 2016;11:887-895.

40. Heidari B, Hosseini R, Javadian Y, Bijani A, Sateri MH, Nouroddini HG. Factors affecting bone mineral density in postmenopausal women. Arch Osteoporos. 2015;10:15.

Turk J Pharm Sci 2026;23(1):50-55
DOI: 10.4274/tjps.galenos.2026.10506

ORIGINAL ARTICLE

Pharmacist-Led Ambulatory Blood Pressure Monitoring in Community Pharmacy: An Operational Feasibility Case Series

Michaela VELLA[1], Francesca WIRTH[1]*, Liberato CAMILLERI[2], Lilian M. AZZOPARDI[1]

[1]University of Malta, Faculty of Medicine and Surgery, Department of Pharmacy, Msida, Malta
[2]University of Malta, Faculty of Science, Department of Statistics and Operations Research, Msida, Malta

ABSTRACT

Objectives: To evaluate the operational feasibility of pharmacist-led ambulatory blood pressure monitoring (ABPM) in community pharmacy practice.

Materials and Methods: This operational feasibility case series recruited eligible participants from four community pharmacies located in three geographical districts in Malta over a three-month recruitment period. Process feasibility included recruitment uptake and participant acceptability. Management feasibility included the completion of a valid 24-hour ABPM recording and successful tracking of post-referral outcomes. Pragmatic post-hoc benchmarks were applied: uptake of at least 60%, completion of valid recordings of at least 80%, and post-referral outcome tracking of at least 80%. Exact 95% confidence intervals (CIs) were calculated using the Clopper-Pearson method.

Results: Ten of 20 invited participants consented, corresponding to an uptake rate of 50% (95% CI, 27.2–72.8%). Valid 24-hour ABPM recordings meeting the pre-defined recording quality criteria were obtained for 9 of the 10 participants, corresponding to a completion rate of 90% (95% CI 55.5–99.7). 4 participants were referred for physician review, and post-referral outcomes were obtained for all 4 participants, corresponding to a tracking rate of 100% (95% CI 39.8–100.0). Three of the 4 referred participants reported an intensification of their antihypertensive treatment.

Conclusion: Pharmacist-led ABPM in community pharmacies demonstrated operational feasibility regarding completion of valid recordings and post-referral tracking, although uptake requires improvement. Participant-reported treatment changes should be interpreted as exploratory signals, and larger implementation studies are warranted.

Keywords: Blood pressure monitoring, ambulatory, community pharmacy services, hypertension, pharmacists

INTRODUCTION

Ambulatory blood pressure monitoring (ABPM) provides repeated blood pressure (BP) measurements over 24 hours in the patient's usual environment and yields 24-hour, daytime and night-time values. ABPM is particularly useful for identifying clinically important phenotypes that cannot be reliably characterised by office BP alone, such as white-coat hypertension, masked hypertension, nocturnal hypertension, and abnormal dipping patterns.[1-7] Compared with office BP, ambulatory BP correlates more strongly with hypertension-mediated target organ damage and predicts cardiovascular events more reliably, making it an important out-of-office method for confirming diagnosis and refining risk stratification.[1-3,4,6]

Current guidance emphasises that ABPM and home BP monitoring (HBPM) are complementary. HBPM is valuable for longer-term follow-up and self-management, whereas ABPM is preferable when confirmation of diagnosis, assessment of apparent resistant hypertension, evaluation of nocturnal BP

***Correspondence:** francesca.wirth@um.edu.mt, **ORCID-ID:** orcid.org/0000-0002-3225-2363
Received: 06.04.2026, **Accepted:** 26.06.2026 **Publication Date:** 13.07.2026

Cite this article as: Vella M, Wirth F, Camilleri L, Azzopardi LM. Pharmacist-led ambulatory blood pressure monitoring in community pharmacy: an operational feasibility case series. Turk J Pharm Sci. 2026;23(1):50-55

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

or clarification of discrepant office readings is required.[1,4,8,9] Although ABPM is well-established for diagnosis and treatment optimisation in specialist care, access in primary care and community pharmacy settings is variable.[10-12] Community pharmacists are accessible healthcare professionals and may be well-placed to support out-of-office BP monitoring services, particularly when embedded within clear referral pathways. The aim was to evaluate the operational feasibility of pharmacist-led ABPM in community pharmacy practice.

MATERIALS AND METHODS

Study design and setting

This was an operational feasibility case series with follow-up. Eligible participants were recruited from four community pharmacies, selected by convenience sampling, located across three geographical districts in Malta.[13] The study evaluated the practical delivery of a pharmacist-led ABPM service and was not designed to test clinical efficacy. Relevant STROBE items were used to guide the reporting of observational data.

Ethical considerations

Approval was obtained from the University of Malta, Faculty of Medicine and Surgery Research Ethics Committee (approval number: MED-2023-00210, dated 19.09.2023). All participants provided written informed consent.

Sample size considerations

A formal sample size calculation was not performed because the study was designed to explore operational feasibility rather than to test a clinical hypothesis. The sample comprised all eligible patients identified during a fixed three-month recruitment period. Exact 95% confidence intervals (CIs) were reported to quantify the uncertainty associated with the resulting small sample.

Eligibility and recruitment

Participants were eligible if they had been diagnosed with hypertension within the previous two months, had experienced a change in antihypertensive medication or dose within that period, or had self-reported non-adherence to antihypertensive therapy. Over the three-month study period, 20 participants met at least one inclusion criterion and were invited to undergo 24-hour ABPM.

Feasibility domains and post-hoc progression benchmarks Feasibility outcomes were organised into three domains. The process domain included uptake, reasons for declining ABPM, and participant-reported tolerability. The management domain included the successful completion of a valid 24-hour ABPM recording and the documentation of post-referral outcomes. The referral domain included potentially abnormal ABPM patterns prompting physician review, and participant-reported actions following that review. Formal progression criteria were not specified in the original approved protocol. To support transparent interpretation and planning of a subsequent implementation study, the following pragmatic post-hoc benchmarks were applied: uptake by at least 60% of invited eligible individuals, completion of a valid 24-hour ABPM recording by at least 80% of consented participants, and documentation of post-referral outcomes by at least 80% of referred participants. The uptake benchmark represented participation of a majority of invited individuals, whereas the completion and tracking benchmarks represented high operational reliability among individuals entering the service pathway. Failure to meet a benchmark was interpreted as indicating an aspect of the service that required modification before larger-scale implementation.

ABPM device and monitoring procedure

Participants attended two appointments with the pharmacist researcher (MV), with the second appointment scheduled 24 hours after the first. At the first appointment, an appropriately sized cuff was fitted to the non-dominant upper arm. BP was initially measured using a calibrated oscillometric upper-arm monitor (A&D® UA-651), followed by fitting GIMA® 24 hours ABPM + pulse rate monitor. The ABPM recording was initiated following confirmation that the readings obtained during fitting were comparable. The ABPM device recorded BP every 30 minutes between 06:00 and 22:00 (daytime) and hourly between 22:00 and 06:00 (nighttime). Participants received verbal instructions from the pharmacist-researcher regarding device handling and activities during the monitoring period. At the second appointment, the ABPM device was removed, and the data were downloaded to generate an individual ABPM report. A recording was considered satisfactory if the minimum criteria for valid readings were met, namely: at least 20 daytime measurements and at least 7 night time measurements.

Device validation and interpretation

The GIMA® 24-hour ABPM monitor used in this study was not listed on the STRIDE BP (an international initiative for the validation of blood pressure measuring devices) registry of validated devices. No published validation data were identified for the specific model used, and it was not possible to confirm whether the device shared its internal measurement algorithm or component design with a validated device from the original equipment manufacturer. Accordingly, the study was framed as an exploratory case series assessing service feasibility. The pharmacist researcher used ABPM outputs to identify potentially abnormal patterns warranting physician review. The physician was responsible for diagnosis and treatment decisions.

Referral workflow and follow-up

The referral process was structured but pragmatic. The pharmacist researcher reviewed the ABPM report, explained the findings to the participant, and provided a printed copy. Participants with potentially abnormal findings were given a written referral note and advised to consult their physician. The pharmacist-researcher did not diagnose hypertension phenotypes, alter medications, or prescribe treatment. The pharmacist-researcher conducted follow-up after the physician consultation to document the participant-reported outcome. Physician actions were not independently verified.

Statistical analysis

Data analysis was descriptive and focused on participant-level and service-process outcomes. Uptake was calculated as the number of individuals who consented divided by the number of individuals invited. Recording completion was calculated as the number who obtained a valid 24-hour recording divided by the number who consented. Referral tracking was calculated as the number of referred participants for whom a post-referral outcome was documented, divided by the total number of referred participants. Two-sided exact 95% CIs for the primary feasibility proportions were calculated using the Clopper-Pearson method. No hypothesis testing, inferential comparisons, or *p*-values were performed. Participant ABPM findings, referral decisions, and reported post-referral outcomes were presented descriptively. Box-and-whisker plots were used as a visual summary of the distributions of daytime and nighttime systolic and diastolic BP readings. Analyses were undertaken using IBM SPSS Statistics version 29.

RESULTS

Recruitment and recording completion

Of the 20 eligible individuals invited to participate, 10 consented, corresponding to an uptake rate of 50% (95% CI: 27.2–72.8). The post-hoc uptake benchmark of at least 60% was not met. Among the 10 individuals who declined, 6 perceived ABPM as burdensome, and 4 felt embarrassed about being seen wearing the device. One participant experienced cuff air leakage, resulting in a device error after 18 hours and yielding only 19 valid daytime readings; hence, this recording was considered invalid. Nine of the 10 consented participants completed valid 24-hour ABPM recordings, corresponding to a completion rate of 90% (95% CI 55.5–99.7).The post-hoc completion benchmark of at least 80% was met.

Participant characteristics

Among the 9 participants with valid recordings, 6 were male and 3 were female. The mean age was 57 years, ranging from 35 to 72 years. The d uration of hypertension was less than 1 year (n = 4), 1 to 5 years (n = 1), 6 to 10 years (n = 3), and 11 to 20 years (n = 1). Seven participants reported no comorbidities; 2 had hypercholesterolaemia, 1 had hypothyroidism, and 1 had depression. 5 participants were receiving antihypertensive monotherapy, and 4 were receiving dual therapy. Antihypertensive medications included thiazide diuretics (n = 4), angiotensin-converting enzyme inhibitors (n = 3), angiotensin II receptor blockers (n = 3), and calcium channel blockers (n = 3).

Tolerability, referral and follow-up

ABPM was reported to be well tolerated by 5 participants. Two participants reported mild bruising, and 2 reported sleep disturbances. The pharmacist researcher's review of the ABPM reports resulted in 4 participants being referred for review by a physician. Participant 1 had normal daytime BP but elevated nocturnal readings. Participants 2 and 4 had elevated BP throughout the 24-hour monitoring period, whereas participant 6 had normal systolic BP but elevated diastolic BP readings (Figure 1). A post-referral outcome was documented for all 4 referred participants, corresponding to a tracking rate of 100% (95% CI 39.8–100.0). The post-hoc referral-tracking benchmark of at least 80% was met. Three of the 4 referred participants reported intensification of antihypertensive treatment. Participants 1 and 4 reported initiating an additional antihypertensive medication, while participant 2 reported a dose increase. Participant 6 reported no change in treatment, but stated that the physician had advised stress reduction. These physician actions were participant-reported and were not independently verified. Participants' profiles and referral outcomes are presented in Table 1.

DISCUSSION

This operational feasibility case series suggests that pharmacist-led ABPM can be delivered in community pharmacy practice, although feasibility varies across the evaluated domains. Valid recordings were obtained for 90% of consented participants, exceeding the post-hoc completion benchmark of 80%, thereby supporting the feasibility of device fitting, participant instruction, 24-hour monitoring, device removal, and report generation. Post-referral outcomes were documented for all referred participants, thereby exceeding the 80%

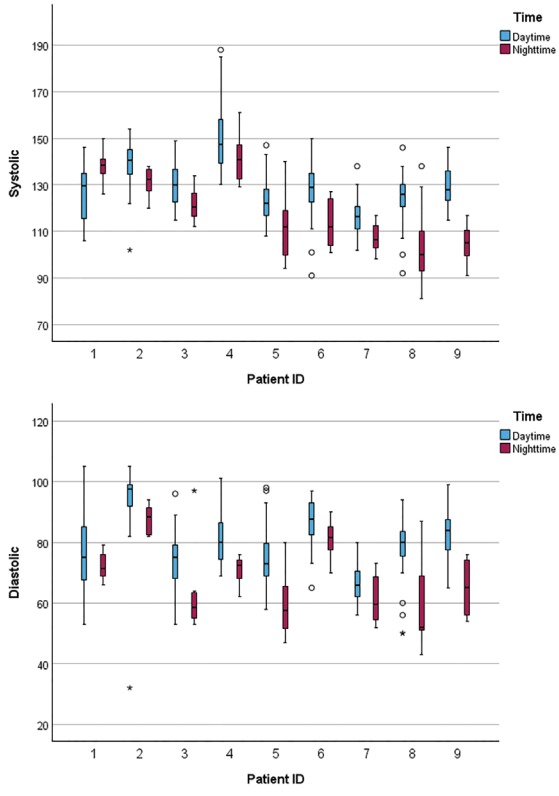


Figure 1. Descriptive distributions of daytime and night-time systolic and diastolic blood pressure readings among the 9 participants with valid 24-hour ABPM recordings. Box-and-whisker plots display the median, interquartile range and outlying values.

ABPM, ambulatory blood pressure monitoring.

Table 1. Individual participant profile and referral outcome for valid 24-hour ABPM recordings (n = 9).

Participant ID	Age/sex and eligibility criterion	Antihypertensive therapy	ABPM values	Pharmacist researcher interpretation	Referral and physician outcome
1	Female/72 years, change in medication/ dose within previous two months	Dual therapy	24-hour mean BP: 131/77 mmHg; daytime mean BP: 129/78 mmHg; night-time mean BP: 138/72 mmHg	Normal daytime BP with elevated nocturnal readings	Referred; participant reported initiation of additional antihypertensive medication
2	Male/52 years, diagnosis of hypertension within previous two months	Monotherapy	24-hour mean BP: 137/93 mmHg; daytime mean BP: 138/94 mmHg; night-time mean BP: 131/88 mmHg	Elevated BP throughout 24-hour period	Referred; participant reported antihypertensive dose increase
3	Female/67 years, change in medication/ dose within previous two months	Dual therapy	24-hour mean BP: 130/73 mmHg; daytime mean BP: 138/94 mmHg; night-time mean BP: 122/63 mmHg	No abnormal referral trigger reported	No physician referral required
4	Male/69 years, diagnosis of hypertension within previous two months	Monotherapy	24-hour mean BP: 149/80 mmHg; daytime mean BP: 150/82 mmHg; night-time mean BP: 141/71 mmHg	Elevated BP throughout 24-hour period	Referred; participant reported initiation of additional antihypertensive medication
5	Female/67 years, diagnosis of hypertension within previous two months	Monotherapy	24-hour mean BP: 121/72 mmHg; daytime mean BP: 123/75 mmHg; night-time mean BP: 112/60 mmHg	No abnormal referral trigger reported	No physician referral required
6	Male/36 years, diagnosis of hypertension within previous two months	Monotherapy	24-hour mean BP: 125/86 mmHg; daytime mean BP: 127/87 mmHg; night-time mean BP: 114/81 mmHg	Normal systolic BP with elevated diastolic readings	Referred; participant reported no treatment change and advice to reduce stress
7	Male/69 years, change in medication/dose within previous two months	Dual therapy	24-hour mean BP: 114/65 mmHg; daytime mean BP: 116/66 mmHg; night-time mean BP: 107/61 mmHg	No abnormal referral trigger reported	No physician referral required
8	Male/44 years, change in medication/dose within previous two months	Dual therapy	24-hour mean BP: 120/74 mmHg; daytime mean BP: 124/77 mmHg; night-time mean BP: 104/61 mmHg	No abnormal referral trigger reported	No physician referral required
9	Male/35 years, diagnosis of hypertension within previous two months	Monotherapy	24-hour mean BP: 125/79 mmHg; daytime mean BP: 130/83 mmHg; night-time mean BP: 105/65 mmHg	No abnormal referral trigger reported	No physician referral required

ABPM, ambulatory blood pressure monitoring; BP, blood pressure.

tracking benchmark. Participant uptake was 50%, below the 60% benchmark, indicating that recruitment and acceptability require modification before larger-scale implementation.

Among participants who completed monitoring, ABPM was generally tolerated consistent with previous reports,[9-11] although mild bruising and sleep disturbance were reported. Half of the eligible individuals nevertheless declined participation because they perceived monitoring as burdensome or felt uncomfortable being seen wearing the device. These findings are consistent with patient-reported concerns described in previous qualitative research and suggest that uptake may be improved through clearer pre-test counselling, reassurance about the purpose and temporary nature of ABPM, discussion of possible discomfort or sleep disturbance, and practical guidance regarding clothing and daily activities during the monitoring period.[14,15]

Four participants were referred because of potentially abnormal BP patterns, including nocturnal hypertension, sustained 24-hour hypertension, and isolated diastolic elevation, of whom three subsequently reported an intensification of antihypertensive treatment. These observations suggest that pharmacist-led ABPM may generate actionable information that prompts medical reassessment. However, the small sample size, self-reported follow-up, and the use of an ABPM device that has not been confirmed as validated limit the strength of clinical inferences.

The measurement validity of the ABPM device has implications for the operational performance of the referral pathway. The specific GIMA® model was not listed on the STRIDE BP registry of validated devices, and no published validation study or confirmed equivalence to a validated original equipment manufacturer device was identified. Measurement uncertainty could result in over-referral, generating avoidable consultations and additional workload for primary care physicians, or under-referral, failing to identify individuals requiring medical review.

Study limitations

This study involved a small convenience sample recruited from four community pharmacies, resulting in wide CIs and limited generalisability. The progression benchmarks were introduced post-hoc to support interpretation and future planning, and were not prospectively established. The ABPM device was not confirmed to be validated, which may have affected the identification of potentially abnormal patterns and the resulting referral decisions. Physician actions reported by participants were not independently verified. Subsequent BP control, persistence of treatment changes, and cardiovascular outcomes were not assessed. In addition, the referral pathway relied on written documentation and patient-mediated physician follow-up rather than direct or integrated electronic communication.

Implications for future research

Future implementation studies should use validated ABPM devices and prospectively specified progression criteria. Recruitment and counselling procedures should be refined to address perceived burden, embarrassment, possible sleep disturbance, and practical concerns that are associated with wearing the device. Larger studies should evaluate patient and healthcare professional experiences, pharmacist training requirements, recording quality, referral appropriateness, clinical effectiveness, and cost-effectiveness. Referral pathways should facilitate direct and secure communication between pharmacists and physicians, and physician actions and subsequent BP outcomes should be independently verified. Integrated models combining ABPM for diagnostic clarification with HBPM for longer-term follow-up may also warrant evaluation.

CONCLUSION

Pharmacist-led ABPM in community pharmacies demonstrated operational feasibility with respect to valid recording completion and post-referral tracking, although uptake requires improvement. Participant-reported treatment changes should be interpreted as exploratory signals, and larger implementation studies are warranted.

Ethics

Ethics Committee Approval: Approval was obtained from the University of Malta, Faculty of Medicine and Surgery Research Ethics Committee (approval number: MED-2023-00210, dated 19.09.2023).

Informed Consent: All participants provided written informed consent.

Footnotes

Authorship Contributions

Concept: F.W., L.M.A., Design: M.V., F.W., L.M.A., Data Collection or Processing: M.V., Analysis or Interpretation: M.V., F.W., L.C., L.M.A., Literature Search: M.V., Writing: M.V., F.W., L.C., L.M.A.,

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: University of Malta Research Grant (PHRRP03).

REFERENCES

1. Cepeda M, Pham P, Shimbo D. Status of ambulatory blood pressure monitoring and home blood pressure monitoring for the diagnosis and management of hypertension in the US: an up-to-date review. Hypertens Res. 2023;46:620-629.
2. Staplin N, de la Sierra A, Ruilope LM, Emberson JR, Vinyoles E, Gorostidi M, Ruiz-Hurtado G, Segura J, Baigent C, Williams B. Relationship between clinic and ambulatory blood pressure and mortality: an observational cohort study in 59 124 patients. Lancet. 2023;401:2041-2050.
3. Kollias A, Kyriakoulis KG, Komnianou A, Stathopoulou P, Stergiou GS. Prognostic value of home versus ambulatory blood pressure monitoring: a systematic review and meta-analysis of outcome studies. J Hypertens. 2024;42:385-392.
4. Lee EM. When and how to use ambulatory blood pressure monitoring and home blood pressure monitoring for managing hypertension. Clin Hypertens. 2024;30:10.

5. Tang A, Yang E, Ebinger JE. Non-dipping blood pressure or nocturnal hypertension: does one matter more? Curr Hypertens Rep. 2024;26:21-30.

6. Kario K, Hoshide S, Mizuno H, Kabutoya T, Nishizawa M, Yoshida T, Abe H, Katsuya T, Fujita Y, Okazaki O, Yano Y, Tomitani N, Kanegae H; JAMP Study Group. Nighttime blood pressure phenotype and cardiovascular prognosis: Practitioner-Based Nationwide JAMP Study. Circulation. 2020;142:1810-1820.

7. Mancia G, Kjeldsen SE. Randomized clinical outcome trials in hypertension. Hypertension. 2024;81:17-23.

8. Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A, Muiesan ML, Tsioufis K, de Pinho RM, Albini FL, Boivin JM, Doumas M, Nemcsik J, Rodilla E, Agabiti-Rosei E, Algharably EAE, Agnelli G, Benetos A, Hitij JB, Cífková R, Cornelissen V, Danser AHJ, Delles C, Huelgas RG, Járai Z, Palatini P, Pathak A, Persu A, Polonia J, Sarafidis P, Stergiou G, Thomopoulos C, Wanner C, Weber T, Williams B, Kjeldsen SE, Mancia G. 2024 European Society of Hypertension clinical practice guidelines for the management of arterial hypertension. Eur J Intern Med. 2024;126:1-15.

9. Jones DW, Ferdinand KC, Taler SJ, Johnson HM, Shimbo D, Abdalla M, Altieri MM, Bansal N, Bello NA, Bress AP, Carter J, Cohen JB, Collins KJ, Commodore-Mensah Y, Davis LL, Egan B, Khan SS, Lloyd-Jones DM, Melnyk BM, Mistry EA, Ogunniyi MO, Schott SL, Smith SC, Talbot AW, Vongpatanasin W, Watson KE, Whelton PK, Williamson JD. 2025 AHA/ACC/AANP/AAPA/ABC/ACCP/ACPM/AGS/AMA/ASPC/NMA/PCNA/SGIM guideline for the prevention, detection, evaluation and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Hypertension. 2025;82:e212-e316.

10. James K, Dolan E, O'Brien E. Making ambulatory blood pressure monitoring accessible in pharmacies. Blood Press Monit. 2014;19:134-139.

11. Dixon DL, Patterson JA, Gatewood S, Kaefer T, Jadallah J, Curtis M, Hawkey L, Grigsby J, Salgado TM, Holdford DA. Development and feasibility of a community pharmacy-driven 24-hour ambulatory blood pressure monitoring service. J Am Pharm Assoc. 2020;60:e332-e340.

12. Omboni S, Khan NA, Kunadian V, Olszanecka A, Schutte AE, Mihailidou AS. Sex differences in ambulatory blood pressure levels and subtypes in a large Italian community cohort. Hypertension. 2023;80:1417-1426.

13. Vella M. Blood pressure monitoring in community pharmacy [doctoral dissertation]. Msida, Malta: Department of Pharmacy, University of Malta; 2024. Available from: https://www.um.edu.mt/library/oar/handle/123456789/130037

14. Carter EJ, Moise N, Alcántara C, Sullivan AM, Kronish IM. Patient barriers and facilitators to ambulatory and home blood pressure monitoring: a qualitative study. Am J Hypertens. 2018;31:919-927.

15. Moosa AS, Kawaja A, Lee EKP, Phoon IKY, Ang ATW, Chang ZY, Lim ACA, Yap J, Huang W, Ng DX, Sng MY, Loh HY, Ng CJ. Feasibility and acceptability of using ABPM to manage hypertension in primary care: a qualitative study. BMC Prim Care. 2026;27:62.