ATATURK
UNIVERSITY
PUBLICATIONS

Pharmata

Official journal of Atatürk University Faculty of Pharmacy

Formerly: International Journal of PharmaATA

Volume 3 • Issue 3 • July 2023

AVES

EISSN 2980-1966

pharmata-ataunipress.org

Pharmata

AVES

Founder
İbrahim Kara

General Manager
Ali Şahin

Finance Coordinator
Elif Yıldız Çelik

Journal Managers
Deniz Kaya
Irmak Berberoğlu
Arzu Arı

Publications Coordinators
Gökhan Çimen
Alara Ergin
İrem Özmen
Derya Azer
Burcu Demirer
Beyza Himmetoğlu Dal
Beril Tekay

Project Coordinators
Doğan Oruç
Sinem Fehime Koz

Project Assistant
Batuhan Kara

Contact
Publisher: Atatürk University
Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES
Address: Büyükdere Cad., 199/6, 34394 Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
E-mail: info@avesyayincilik.com
Webpage: www.avesyayincilik.com

Pharmata

Pharmata

AIMS AND SCOPE

Pharmata is a scientific, open access, online-only periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is an official publication of the Atatürk University, and published quarterly in January, April, July, and October. The publication language of the journal is English.

Pharmata aims to contribute to the literature by publishing manuscripts at the highest scientific level. The journal publishes research articles, reviews and short communications that are prepared in accordance with ethical guidelines.

The scope of the journal includes but not limited to pharmaceutical analysis of complex system, quality control and methods of biotech drugs, action mechanism and their metabolisms in body, quantitative and qualitative analysis in the drug screening process, molecular pharmacology, biopharmaceutics, pharmacognosy, pharmaceutical botany, pharmaceutical technology studies, clinical laboratory and bioanalysis, the pharmacological studies, the toxicologic studies, analytical chemistry techniques and methods, new biochemistry methods for pharmaceutical analysis, rapid screening methods, new analytical techniques and methods, pharmaceutical chemistry, synthesis and analysis new drug molecules, others: pharmaceutical solid materials (including biomaterials, polymers and nanoparticles) biotechnology products (including genes, peptides, proteins and vaccines) engineered cells.

The target audience of the journal includes researchers and specialists who are interested or working in all fields in the journal's scope.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Pharmata currently indexed in EBSCO.

All expenses of the journal are covered by the Atatürk University. Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://www.pharmata-ataunipress.org. The journal guidelines, technical information, and the required forms are available on the journal's web page.

You can find the current version of the Instructions to Authors at https://pharmata-ataunipress.org/.

Editor in Chief: Yücel KADIOĞLU
Address: Department of Analytical Chemistry, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey
E-mail: yucel@atauni.edu.tr

Publisher: Atatürk University
Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES
Address: Büyükdere Cad., 199/6, 34394 Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
E-mail: info@avesyayincilik.com
Webpage: www.avesyayincilik.com

Pharmata

CONTENTS

RESEARCH ARTICLES

REVIEWS

ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2023.23010

Effect of Tryptanthrin Compound on Lipopolysaccharide-Induced Inflammation Model in L929 Cell Line

Mehmet YILMAZ
Zerrin KUTLU

Department of Biochemistry, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey

ABSTRACT

Objective: Our aim is to evaluate the effects of the tryptanthrin compound, with antimicrobial and anti-inflammatory effects, on the lipopolysaccharide inflammation model created in the L929 cell line.

Methods: L929 cell lines were placed in a 5% CO_2 incubator at 37°C, using standard cell culture procedures, and lipopolysaccharide was applied with Prn pure substance with a final concentration of 2 µL and 1 µL after 1 hour. Following the lipopolysaccharide application, the (3- [4,5- dimethylthiazol-2-yl] -2,5-diphenyl tetrazolium bromide protocol, a colorimetric method, was applied to determine cell viability at the end of the required 24th, 48th, and 72nd hours incubation times.

Results: IC_{50} values were calculated as 0.3 µg/mL for tryptanthrin and 1 µg/mL for lipopolysaccharide . Application of lipopolysaccharide to L929 cell lines caused a significant decrease in cellular index depending on time. Also, in the tryptanthrin + lipopolysaccharide groups, it was found that the decreased cell index significantly increased even closer to the control compared to the lipopolysaccharide applied group. It was found that the inflammation and cell damage caused by lipopolysaccharide applied to the L929 cell line improved after tryptanthrin application.

Conclusion: When we look at the results of our study as a whole, it was determined that the viability rates of the cells were above 85% as a result of the tryptanthrin application and it was significantly above the 70% viable cell ratio recommended by ISO 10993-5: 2009 Dın en ISO (2009) for cytotoxicity.

Keywords: Inflammation, L929, LPS, MTT, Tryptanthrin

INTRODUCTION

Tryptanthrin (TRP) is indeed a natural compound found in indigo plants, and it belongs to the class of indole quinazoline alkaloids. Researchers have been studying the effects of TRP due to its various biological and pharmacological activities. One notable area of investigation is the antimicrobial activity of TRP. Studies have shown that TRP exhibits antimicrobial properties against a range of microorganisms, including bacteria, fungi, and parasites. It has demonstrated efficacy against both Gram-positive and Gram-negative bacteria, making it a potentially valuable compound for the development of new antimicrobial agents. Tryptanthrin has also been found to possess anti-inflammatory properties. Inflammation is a natural response of the immune system, but excessive or chronic inflammation can lead to various diseases. Tryptanthrin has been shown to inhibit the production of pro-inflammatory molecules and suppress the activation of inflammatory pathways, thus potentially providing a therapeutic benefit in conditions characterized by inflammation. Additionally, TRP has shown antiallergic activity. Allergic reactions occur when the immune system overreacts to harmless substances. Tryptanthrin has been found to inhibit the release of histamine, a key mediator of allergic reactions, from mast cells. This action may help alleviate allergic symptoms and provide relief for individuals with allergies. It is worth noting that while TRP shows promise in these areas, further research is still needed to fully understand its mechanisms of action and explore its potential applications in medicine. Researchers are also interested in investigating the synergistic effects of TRP with other compounds found in plants, as well as its potential side effects and safety profile.[1]

Lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria, and it is commonly used to induce an inflammatory response in research studies. Lipopolysaccharide has the ability to activate macrophages, which are immune cells involved in the innate immune response. When macrophages are exposed to LPS, it triggers a series of signaling pathways within these cells. Three important pathways that are activated in response to LPS are the NF-κB (nuclear factor kappa-ligh

Received: 26.06.2023
Accepted: 04.07.2023
Publication Date: 31.07.2023

Corresponding Author:
Zerrin KUTLU
E-mail: kutluzerrin@atauni.edu.tr

Cite this article as: Yılmaz M, Kutlu Z. Effect of tryptantrhin compound on lipopolysaccharideinduced inflammation model in L929 cell line. *Pharmata*, 3(3), 54-58.

t-chain-enhancer of activated B cells), MAPK (mitogen-activated protein kinase), and JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathways. The NF-κB pathway is a key regulator of immune and inflammatory responses. Activation of this pathway leads to the production of pro-inflammatory cytokines, chemokines, and other molecules that promote inflammation. The MAPK pathway is involved in cellular processes such as cell proliferation, differentiation, and inflammation. Lipopolysaccharide activates MAPK signaling, which can lead to the production of pro-inflammatory mediators and the regulation of immune responses. Macrophages secrete growth factors, including transforming growth factor-beta (TGF-β), which can stimulate fibroblast proliferation and activation. This interaction between macrophages and fibroblasts is crucial for tissue repair and wound-healing processes. Overall, the activation of macrophages by LPS and their subsequent signaling pathways play a vital role in the inflammatory response and immune regulation. Understanding these processes can help in the development of therapeutic strategies for various inflammatory and immune-related conditions.[2]

Inflammation is a vital defense mechanism of the immune system. It is a complex biological response that can be triggered by a variety of factors, including damaged cells, toxic compounds, and invading pathogens. The primary purpose of inflammation is to protect the body from harmful stimuli and initiate the healing process. When tissues are damaged or infected, the immune system recognizes these threats and responds by mobilizing immune cells and releasing signaling molecules.

Inflammation is typically characterized by the classic signs of redness, heat, swelling, pain, and sometimes loss of function in the affected area. These symptoms arise due to increased blood flow, increased permeability of blood vessels, and the influx of immune cells and fluid into the tissues. While acute inflammation is an important and necessary response for maintaining health, chronic inflammation can be harmful. Prolonged or unresolved inflammation can lead to tissue damage and contribute to the development of various chronic conditions, including autoimmune disorders, cardiovascular diseases, and certain cancers. Therefore, maintaining a balanced inflammatory response is crucial for overall health.[3]

It is important to note that the specific details of the inflammatory response can vary depending on the type of tissue involved and the nature of the stimulus. However, the fundamental steps you mentioned—recognition of stimuli, activation of pathways, release of mediators, and activation of inflammatory cells—are indeed central to the inflammatory response in all tissues (Figure 1).[4]

Phagocytes, including neutrophils, macrophages, and dendritic cells, are key players in the inflammatory response and have pathogen recognition receptors (PRRs) on their surfaces. These PRRs recognize specific molecular motifs on pathogens, known as pathogen-associated molecular patterns (PAMPs). Cytokines, such as interleukins and tumor necrosis factor-alpha (TNF-α), serve as signaling molecules that mediate and amplify the immune response. In addition to cytokine release, the activation of intracellular signaling pathways is a crucial step in the inflammatory response. The JAK-STAT pathway is involved in cytokine signaling and regulates gene expression. Activation of this pathway can lead to the production of specific cytokines and the modulation of immune cell functions. These signaling pathways work in coordination to amplify the inflammatory response

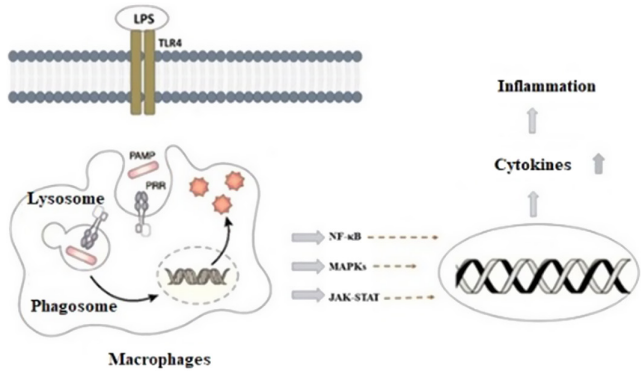

Figure 1. The general mechanism of LPS-induced inflammation

and regulate the expression of genes involved in immune and inflammatory processes. Understanding the activation of these pathways and the release of proinflammatory molecules is crucial for unraveling the complex mechanisms underlying inflammation and designing targeted interventions for inflammatory diseases.[5]

In the process of inflammation, various plasma proteases from systems such as the kinin-kallikrein, complement, coagulation, and fibrinolytic systems are activated. Activation of these proteases leads to the generation of specific products that contribute to the inflammatory response. Neutrophils are the first responders and play a crucial role in combating pathogens. They are attracted to the inflammatory site and migrate across the blood vessel walls to reach the site of infection or tissue damage. Macrophages, as you mentioned, are another important cell type involved in inflammation. Macrophages are versatile immune cells that reside in tissues, including inflamed tissues. They play a vital role in protecting the body against pathogens through various mechanisms, one of which is phagocytosis. Macrophages recognize and engulf pathogens through phagocytosis, internalizing them into specialized compartments where they can be destroyed. Furthermore, macrophages contribute to the inflammatory response by releasing additional inflammatory mediators and cytokines, amplifying the immune response and coordinating the activities of other immune cells. Together, the activation of plasma proteases, the resulting products, changes in vascular permeability, recruitment of neutrophils, and the role of macrophages all contribute to the complex process of inflammation and help the body combat pathogens and initiate the healing process.[6] Interleukin-1β (IL-1β), interleukin-6 (IL-6), and TNF-α are key cytokines produced by activated macrophages. Interleukin-1β, IL-6, and TNF-α have various roles in the inflammatory response. The release of lipid-based mediators and cytokines by activated macrophages allows them to communicate with other immune cells and coordinate the inflammatory response. Therefore, understanding the regulation of these molecules and their functions is crucial for developing targeted therapeutic approaches for inflammatory conditions.[7]

It is fascinating to see your interest in investigating the effects of TRP in the LPS-induced inflammation model using the L929 cell line. Indeed, fibroblasts play a crucial role in the inflammatory process, and their activation and interaction with other cells contribute to the overall inflammatory response and tissue repair. As you mentioned, fibroblasts act as sentinel-like cells in tissues and are among the first to respond to damage signals. They contribute to maintaining the structural integrity of connective

tissues by promoting immune cell adhesion and secreting components of the extracellular matrix that support collagen protein and adjacent cells.[2] Given the potential therapeutic benefits of plant-derived compounds and their anti-inflammatory properties, exploring the effects of TRP in the LPS-induced inflammation model is a valuable approach. The LPS-induced inflammation model is widely used because it triggers macrophage activation and subsequent activation of multiple inflammatory pathways.[7]

Using the L929 cell line, you can investigate the effects of TRP in this model and assess its impact on the inflammatory response. This approach can provide insights into the anti-inflammatory potential of TRP and its mechanisms of action, shedding light on its therapeutic potential. By examining the effects of TRP in the LPS-induced inflammation model, you can contribute to the understanding of plant-based compounds as potential therapeutic agents for inflammation-related conditions. It is an exciting area of research that can pave the way for the development of new treatments or interventions targeting inflammation. Remember to carefully design your experiments, control for variables, and analyze your results rigorously to draw meaningful conclusions.

METHODS

Cell Lines

In this study, the L929 cell line was used to evaluate the effects of the TRP compound on LPS-induced inflammation-induced cell damage in the L929 (ATCC® CRL-2091) cell line. The L929 cell line was purchased from the American Type Culture Collection (ATCC, USA) and used by reproducing from existing stocks available in the Cell Culture Laboratory, affiliated with the Department of Medical Pharmacology, Ataturk University School of Medicine.

Experimental Protocol and Details

This study was carried out in the Cell Culture Laboratory of the Department of Medical Pharmacology, Faculty of Medicine, Ataturk University. First, different concentrations of doses were determined to calculate the IC_{50} value of the TRP compound and LPS (Table 1).

After the cells preserved in Cryotube in the liquid nitrogen tank were dissolved, they were planted in flasks of T75 cm^2 (Gibco®, UK) with filtered cap. After the cells were determined with the help of Inverted (Leica®, Germany) light microscope (20×-40×), where 85%-90% of them became confluent (20×-40×), 5×10^3 cells were sown in each well of 96-well microplates. After the TRP and LPS applications, the colorimetric method (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) protocol) was applied.

Preparation of Tryptanthrin and Lipopolysaccharide

Since the TRP compound is insoluble in water, 1% dimethyl sulfoxide was added to dissolve the substance after vortexing. Different concentrations (20 µg/mL; 15 µg/mL; 10 µg/mL; 7.5 µg/mL; 5 µg/mL; 2.5 µg/mL; 1 µg/mL) were prepared after passing through the sterile filter (CAS: 13220-57-0, 97%, MW:248.24, BLDpharm, UK) with a sol pore diameter of 0.2 µm . Lipopolysaccharide (*E. Coli* O55: B5, Sigma-Aldrich®, St. Louis, USA) in lyophilized form was dissolved in pure water to prepare different concentrations.

Table 1. Doses Used to Calculate IC_{50} Values of Lipopolysaccharide and Tryptanthrin

LPS	TRP
Concentrations	
20 µg/mL	20 µg/mL
15 µg/mL	15 µg/mL
10 µg/mL	10 µg/mL
7.5 µg/mL	7,5 µg/mL
5 µg/mL	5 µg/mL
2.5 µg/mL	2,5 µg/mL
1 µg/mL	1 µg/mL

LPS, lipopolysaccharide; TRP, tryptanthrin.

Cell Culture Studies

According to cell culture studies, the L929 cell line is cultured in a medium consisting of 89% DMEM (Dulbecco's modified eagle medium), 10% FBS (fetal bovine serum), 1% PSA (penicillin–streptomycin–amphotericin B), and 2% L-glutamine. These components provide the necessary nutrients, growth factors, and antibiotics to support cell growth and maintain cell viability.

The cells are proliferated using standard cell culture procedures in a 5% CO_2 incubator at 37°. This temperature and CO_2 concentration create an optimal environment for cell growth and maintenance. To determine cell density and viability, the cells are examined using an inverted light microscope, such as the Leica microscope. Once the cells reach a confluent state with 85%-90% density, the trypan blue exclusion method is used to assess cell viability.

In this method, a mixture of 100 µL trypan blue, 90 µL PBS, and 10 µL medium containing cells is prepared in a microcentrifuge tube. A small volume (10 µL) of this mixture is then transferred to a cell counting device, such as the INNOVATIS device, which allows for accurate cell counting and viability determination. After performing the necessary calculations, the cells are seeded into the experimental wells. It is stated that 5×10^3 cells are seeded in each well, with the number of groups multiplied by 5×10^3 to ensure consistent cell seeding across all groups. These details provide a clear understanding of the cell culture conditions and procedures used for the L929 cell line. Following proper cell culture techniques and accurately determining cell density and viability are crucial for reliable experimental outcomes.

Evaluation of Cell Viability with the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide Method

To summarize the experimental procedure:

1. L929 cell lines were cultivated in 3 microplates, with 5×10^3 cells (5000 cells) seeded in each well, using a standard cell culture medium.
2. The microplates were placed in a 5% CO_2 incubator at 37° for 24 hours to allow the cells to adhere and grow.
3. After the 24-hour incubation, the TRP compound was applied at the predetermined doses. The final concentration of the TRP compound was 2 µL.
4. Following the TRP application, the LPS was applied after 1 hour at a final concentration of 1 µL.
5. After the application of LPS, the cells were further incubated for 24, 48, and 72 hours to allow for the desired incubation times.
6. At the end of each incubation time (24th, 48th, and 72nd hours), the cytotoxic effects were assessed using the MTT assay, a colorimetric method. The MTT assay measures the metabolic activity of cells, which is indicative of cell viability.
7. The % viable cell ratio was calculated using the following equation:
   - % viable cell ratio = (absorbance of treated sample/absorbance of control) × 100
   - The absorbance values were obtained using a spectrophotometer or a microplate reader, and the control sample represents the untreated cells.

- By applying the MTT assay, determine the cytotoxic effects of the TRP compound and LPS on the L929 cells at different time points can be determined. This assay provides information on cell viability and can help assess the impact of the compounds on cell health.
- The MTT assay protocol provided by the manufacturer (Roche, Cat. No. 11 465 007 001) should be followed for accurate and reliable results. This protocol typically involves the addition of MTT reagent, formazan crystal dissolution, and absorbance measurement.
- By analyzing the % viable cell ratio, the potential cytotoxic or protective effects of the TRP compound and LPS on the L929 cells over time can be evaluated. This information can contribute to understanding the cellular response to these substances and their potential therapeutic implications.

$$\%\,\text{Percentage viability} = \frac{(\text{Compound} - \text{treated cell absorbance}) - (\text{absorbance of the empty well})}{(\text{control cell absorbance} - \text{absorbance of the empty well})} \times 100$$

Statistical Analysis

The data were assessed statistically utilizing Statistical Package for Social Sciences (SPSS) software 22.00 (IBM, USA). It was conducted utilizing a Tukey test, which comprised one-way analysis variance and post hoc multiple comparative assays.

RESULTS

IC_{50} Values of Lipopolysaccharide and Tryptanthrin

To determine the most effective dose on the L929 cell line, TRP and LPS different concentrations were applied. IC_{50} values that inhibit the proliferation of 50% of different concentrations of TRP and LPS on the L929 cell line in vitro were determined (Table 1).

IC_{50} values were calculated as 0.3 µg/mL for TRP and IC_{50} for LPS as 1 µg/mL using the logarithmic transformation of TRP and LPS concentration and nonlinear regression sigmoidal dose–response analysis (GraphPad 8.0 Prism, La Jolla, Calif, USA).

Results of Cell Viability

Based on the results obtained, it appears that the TRP compound has a protective effect on L929 cells against inflammation-induced damage caused by LPS. The LPS group showed a significant decrease in live-cell ratio compared to the control group, indicating that LPS-induced cytotoxicity in the L929 cells. Treatment with different doses of TRP pure substance (ranging from 20 µg/mL to 1 µg/mL) resulted in increased cell proliferation, with the highest live-cell ratio observed at a dose of 5 µg/mL.

At the 24th hour, the LPS group exhibited a live-cell ratio of 61.29%. The TRP group showed a significantly higher live-cell ratio of 90.81%, and the TRP+LPS group had a live-cell ratio of 83.01%. (Table 2)

At the 48th hour, the live-cell ratio in the TRP group decreased to 43.75%. However, in the LPS group, the live-cell ratio was significantly lower at 27.38%. The TRP+LPS group showed a higher live-cell ratio of 84.40%.

At the 72nd hour, the live-cell ratio in the TRP group increased to 92.10%. The TRP+LPS group had a slightly lower live-cell ratio of 88.21%, but it was still significantly higher than the LPS group (12.91% live cells). (Table 2)

Overall, the TRP compound was found to prevent inflammation-related cell damage in the L929 cell line induced by LPS. The cell viability rate in the TRP+LPS group was significantly higher than in the LPS group at all time points (24th, 48th, and 72nd hours) (Table 2).

The findings suggest that TRP promotes cell proliferation and helps protect L929 cells from inflammation-induced damage. The cell viability rates observed in all time periods (above 92%) exceed the recommended 70% rate for cytotoxicity according to ISO 10993-5:2009 guidelines.

These results support the potential use of TRP as a therapeutic agent for mitigating inflammation-induced cell damage and promoting cell viability in the L929 cell line. Further studies may be warranted to explore the underlying mechanisms of TRP's protective effects and its potential applications in inflammatory conditions.

DISCUSSION

The study conducted investigated the effects of the TRP compound, isolated from the root of Ferulago pauciradiata plant, on LPS-induced inflammation-induced cell injury in the L929 cell line. The results of the study support the hypothesis that the TRP compound can prevent inflammation and reduce cell damage. Throughout the study, it was observed that the TRP compound had a protective effect on the L929 cells. At 24th hours, 48th hours, and 72nd hours, the TRP compound prevented inflammation-induced cell injury and increased cell proliferation. These effects brought the cell viability closer to that of the control group, indicating a positive impact of the TRP compound on cell health. The results obtained from this study are consistent with the hypothesis that the TRP compound can reduce inflammatory cell damage. This suggests that the TRP compound derived from the root of *Ferulago pauciradiata* plant may have the potential as a therapeutic agent for mitigating inflammation and promoting cell viability. Further research and investigations may be needed to better understand the mechanisms of action and potential applications of the TRP compound in treating inflammation-related conditions.

The inflammatory response is the natural defense system of the host against pathogens in general, and inflammatory cells and macrophages are activated in this process.[9] Lipopolysaccharide, which is a component of the cell wall of Gram-negative bacteria, is often used in the inflammatory response due to its ability to activate macrophages.[10] The induced macrophages strengthen the inflammatory response by destroying the pathogen with the various juices they secrete and reshaping damaged tissue structures. Pharmacological reduction of LPS-induced inflammatory mediators is considered one of the attractive therapeutic strategies for acute and chronic inflammatory diseases.[8] In the inflammatory process, food components with anti-inflammatory properties may attract attention, provided they can be preserved

Table 2. Results of Cell Viability According to Groups and Hours

Hours	LPS	TRP	TRP+LPS
Groups			
24th	61.29%	90.81%	83.01%
48th	27.38%	43.74%	84.40%
72nd	12.91%	92.10%	88.21%

LPS, lipopolysaccharide; TRP, tryptanthrin.

in the digestive process.[11] In recent years, natural products and their synthetic derivatives have been gaining increasing popularity as potential drugs for the treatment of various diseases affecting humans.[12] Also, it is known that many plants show anti-inflammatory activity based on folk medicine knowledge. Therefore, researchers turn to plants for new therapeutic agents.

Tryptanthrin which is present in indigo plants is an indole quinazoline alkaloid. Many studies have revealed that TRP has many biological and pharmacological activities such as antimicrobial, anti-inflammatory, and antiallergic ones.[1] In accordance with the results of the studies conducted, while the effects of TRP, which is a coumarin derivative, on L929 cells at the 24th, 48th, and 72nd hours did not show any toxic effect compared to the control group, it was determined that TRP application increased the amount of decreased cell index in LPS groups.

As a result, studies[12-15] found that coumarins have a proliferative effect. When LPS was applied 1 hour after applying different doses of the TRP compound it was found that as the concentration of the TRP pure substance increased, the cell proliferation increased and the highest viable cell ratio was observed at a dose of 8 µg/mL.

When we look at the results of our study as a whole, it was determined that the viability rates of the cells were above 85% as a result of the TRP application and it was significantly above the 70% viable cell ratio recommended by ISO 10993-5: 2009 Dın en ISO (2009) for cytotoxicity.

Ethics Committee Approval: There is no 'Ethics committee approval because this is a cell culture study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.Y., Z.K.; Design – M.Y., Z.K.; Supervision – M.Y., Z.K.; Resources – M.Y., Z.K.; Materials – M.Y., Z.K.; Data Collection and/or Processing – M.Y., Z.K.; Analysis and/or Interpretation – M.Y., Z.K.; Literature Search – M.Y., Z.K.; Writing Manuscript – M.Y., Z.K.; Critical Review – M.Y., Z.K.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

REFERENCES

1. Popov A, Klimovich A, Styshova O, et al. Design, synthesis and biomedical evaluation of mostotrin, a new water soluble tryptanthrin derivative. *Int J Mol Med*. 2020;46(4):1335-1346. [CrossRef]
2. Moeed A, Michael M, Emma GM. Fibroblast activation and inflammation in frozen shoulder. *Plus One*. 2019:1-16.
3. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2018;9(6):7204-7218. [CrossRef]
4. Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. *Biochim Biophys Acta*. 2005;1754(1-2):253-262. [CrossRef]
5. Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. *Nat Immunol*. 2017;18(8):826-831. [CrossRef]
6. Xu J, Zhao Y, Aisa HA. Anti-inflammatory effect of pomegranate flower in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. *Pharmaceutical Biology*. 2017;55(1):2095-2101. [CrossRef]
7. Shao J, Li Y, Wang Z. A novel naphthalimide derivative, exhibited anti-inflammatory effects via targeted-inhibiting TAK1 following down-regulation of ERK1/2- and p38 MAPK-mediated activation of NF-kB in LPS-stimulated RAW264.7 macrophages. *Int Immunupharmacol*. 2013;17:216-228.
8. Zhang S, Ma J, Sheng L, et al. Total Coumarins from Hydrangea paniculata show renal protective effects in lipopolysaccharide-induced acute kidney injury via anti-inflammatory and antioxidant activities. *Front Pharmacol*. 2017;8:872. [CrossRef]
9. Vysakh A, Prasad G, Kuriakose J. Rotula aquatica Lour attenuates secretion of proinflammatory mediators and cytokines in lipopolysa ccharide-induced inflammatory responses in murine RAW 264.7 macrophages. *Inflammopharmacology*. 2018;26:29-38.
10. Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cell Signal*. 2001;13(2):85-94. [CrossRef]
11. Bistrian B. Systemic response to inflammation. *Nutr Rev*. 2007;65(12 Pt 2):S170-S172. [CrossRef]
12. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev*. 2009;22(2):240-273. [CrossRef]
13. Neumann U, Louis S, Gille A, et al. Anti-inflammatory effects of Phaeodactylum tricornutum extracts on human blood mononuclear cells and murine macrophages. *J Appl Phycol*. 2018;30(5):2837-2846. [CrossRef]
14. Mustafa YF, Najem MA, Tawffiq ZS. Coumarins from creston apple seeds: isolation, chemical modification, and cytotoxicity study. *J Appl Pharm Sci*. 2018;8(08):49-56.
15. Kasumbwe K., Kabange N., Venugopala N. 2017 Synthetic Mono/dihalogenated Coumarin Derivatives and Their Anticancer Properties. Anti-Cancer Agents in Medicinal Chemistry, 17(2),276-85.

ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2023.23008

Development and Validation of HPLC-UV Method for Determination of Meloxicam in Tablet Dosage Formulation

Reyhan Sıla ÇELİK
Burak BAYRAK
Yücel KADIOĞLU

Department of Analytical Chemistry, Atatürk University, Faculty of Pharmacy, Erzurum Turkey

ABSTRACT

Objective: The development and validation of a novel, simple, and quick high-performance liquid chromatography–ultraviolet detection (HPLC-UV) technique for measuring meloxicam in pharmaceutical formulations was made.

Methods: The technique parameters were tuned to be 0.8 mL/min flow rate, variable column temperature, 290 nm wavelength, 10 µL injection volume, and a mobile phase combination of water (with 0.6% triflour acetic acid—pH:2.6) and methanol (30 : 70 v/v) to carry out this study. In this study, valsartan was used as ınternal standard (IS).

Results: Specificity, the limit of quantitation (LOQ), linearity, accuracy, precision, stability, recovery, and ruggedness were all tested. The technique was linear between 1.0 µg/mL and 50 µg/mL, with precision (relative standard d (RSD) %) and accuracy (relative error %) of less than 3.9% and 0.7%, respectively. The LOQ and LOD values of method were 1.00 and 0.25 µg/mL, respectively. Analytical recovery from pharmaceutical preparations was performed according to the standard addition method, and the average analytical recovery value was determined as 100.4%. The developed and validated HPLC-UV method was successfully applied to 4 commercial tablet dosage formulations obtained from a local pharmacy store in Turkey (Zeloxim, Melox, Meksun, Exen).

Conclusion: It has been concluded that the developed HPLC-UV method is sensitive, accurate, and precise and can be successfully applied in quality control studies in the pharmaceutical industry.

Keywords: HPLC-UV, meloxicam, tablet dosage formulation

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), which are non-narcotic analgesics, are also referred to as simply anti-inflammatory drugs, which better suit their pharmacological profile. They are also known as non-opioid analgesics. The anti-inflammatory efficacy of this group of drugs is weaker compared to the most potent synthetic or natural anti-inflammatory steroid drugs known as glucocorticoids. Their analgesic activity is generally weaker compared to strong analgesics that do not possess anti-inflammatory effects, such as narcotic analgesics. However, they are preferably used in most painful conditions due to their non-addictive properties and their lack of causing narcotic-like effects such as sedation and clouding of consciousness.[1-3]

Meloxicam is one of the NSAIDs and is commonly used for pain, inflammation, and fever control. Meloxicam is an yellow crystalline powder. Its molecular weight is 351.39 g/mol. The melting point is 242-250°C. Meloxicam has a chemical structure of $C_{14}H_{13}N_3O_4S_2$ and a molecular weight of 351.403 g/mol. Its IUPAC name is (8E)-8-hydroxy-[(5-methyl-1,3-thiazol-2-yl)amino]methylidene]-9-methyl-10,10-dioxo-10-thia-9-azabicyclo [4.4.0]dec - 1,3,5-triene-7-one. The chemical structure is shown in Figure 1.[4]

Meloxicam is insoluble in water but soluble in dimethyl sulfide, dimethyl sulfoxide, and alcohols. It is partially and slowly absorbed from the gastrointestinal tract. It reaches its peak in plasma approximately 5-6 hours after a single dose.[5] The elimination half-life is about 20 hours. Comparative trials lasting between 23 days and 1 month have shown that gastrointestinal side effects occur at the frequency seen with placebo and at a lower rate than those who took piroxicam 20 mg per day or diclofenac 100 mg per day.[6] However, it has been reported that the analgesic effect may be slightly lower. It is given orally at a dose of 7.5 mg once a day during the meal, increasing the daily dose to 15 mg if necessary. It is mainly used for osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.[7]

Received: 11.07.2023
Accepted: 03.07.2023
Publication Date: 31.07.2023

Corresponding Author:
Reyhan Sıla ÇELİK
E-mail: reyhansilakadioglu@hotmail.com

Cite this article as: Çelik RS, Bayrak B, Kadıoğlu Y. Development and validation of HPLC-UV method for determination of meloxicam in tablet dosage formulation. *Pharmata* 2023;3(3):59-63.

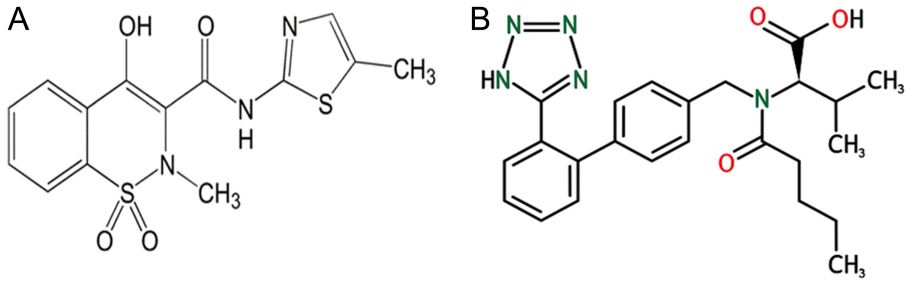

Figure 1. The chemical structures of meloxicam (A) and valsartan (B).

High-performance liquid chromatography (HPLC) is an analytical method used to separate and quantify components in a mixture. An HPLC device basically consists of a pump, column, and detector. The separation is carried out by using various mobile phases over the stationary phase used. The detector can be a variety of types, including UV/visible absorbance detectors, fluorescence detectors, or mass spectrometers. Among the advantages of the method are parameters such as wide usage areas and high sensitivity.

In the literature, several analytical methods have been reported for the quantification of meloxicam in bulk and tablets. These methods include spectrophotometric techniques,[8-11] near infrared spectrometry,[12] capillary zone electrophoresis,[13] HPLC,[14-20] and HPTLC/TLC.[21,22] Based on the methods reported above, it was aimed to develop a simple, fast, and accurate HPLCUV method for the determination of meloxicam in tablets. In this study, HPLC-UV method was developed and validated in the analysis of meloxicam in tablets without derivatization. The developed and validated method was successfully applied to 4 different commercial tablets for meloxicam analysis.

METHODS

Reagents and Chemicals

Meloxicam and valsartan [internal standard (IS)] were obtained from Novagenix Bioanalytical Pharmaceutical Research and Development Center San. ve Tic. Inc. The trifluoro acetic acid (TFA, analytical grade) and methanol (LC grade) were purchased from Merck (Germany). The deionized water that was made fresh every day, filtered (0.45 m) was used. Four commercial tablets (Meksun, Exen, Zeloxim, and Melox) containing the active ingredient meloxicam were obtained from the Turkish pharmaceutical market.

Instrumentation and Conditions for Chromatography

The HPLC System (Agilent Technologies 1200 Series) with a UV detector (Agilent Technologies), degasser (Agilent Technologies), pump (Agilent Technologies), auto-sampler (Agilent Technologies), and computer (HP).

Table 1. Method Conditions Used Proposed Study

Conditions	Meloxicam
Column	C_{18} (250 × 4.6 mm, 5 µm)
Detector	UV
Wavelength	290 nm
Mobile phase	Methanol : water with 0.6 TFA (70 : 30, v/v; pH: 2.6)
Flow rate	0.8 mL/min
Column temperature	Variable temperature
Injection volume	10 µL
Internal standard and concentration	Valsartan and 5 µg/mL

TFA, trifluoro acetic acid.

The most important aspect to focus on when developing a liquid chromatographic method is to determine if sufficient separation has been achieved. The selectivity of the chromatographic system reflects all interactions between the solutes, mobile phase components, and stationary phase. These interactions can be managed by modifying experimental conditions such as temperature, flow rate, column, and mobile phase composition. In the HPLC study, the chromatographic method conditions applied for the active ingredient meloxicam are provided in Table 1.

Preparation Stock, Standard, and Quality Control Solutions

The meloxicam stock solution was prepared by weighing the meloxicam standard on a sensitive balance and dissolving it in a 100 mL volumetric flask with methanol. All prepared solutions were kept in the refrigerator at +4°C until analysis. From this prepared stock solution, appropriate amounts were taken and diluted with methanol to prepare standard working solutions at concentrations of 1, 5, 10, 20, 30, 40, and 50 µg/mL, and quality control solutions at concentrations of 2, 25, and 45 µg/mL. The internal standard working solution at a concentration of 5 µg/mL was prepared from the valsartan standard substance.

Preparation of Tablet Solutions

Eight tablets were taken from each of the Exen tablet formulation containing 15 mg meloxicam and the Meksun, Zeloxim, and Melox tablet formulations containing 7.5 mg meloxicam, and the weights of the tablets were determined. 8 tablets taken were ground in a mortar until they turned into powder and thoroughly mixed. From this mixture, an amount equivalent to the average weight of 1 tablet was weighed according to the sampling method. It was transferred to a 100 mL volumetric flask, and methanol was added to dissolve it while being mixed on a vortex mixer. After filtration, the volume was adjusted to 100 mL with methanol. Suitable volumes were taken from this solution to prepare tablet solutions at a concentration of 5 µg/mL, and they were injected into the HPLC system for analysis.

Mobil Phase Optimization

The composition of the mobile phase plays a significant role in the retention of compounds in reversed-phase liquid chromatography. The polarity of the solvent mixture used as the mobile phase is a measure of its eluting power and is a fundamental factor that affects the retention of the analyte in reversed-phase HPLC. In this study, solvent mixtures of methanol–water (with 0.6% TFA) and acetonitrile–water (with 0.6% TFA) were tested as mobile phases, and the methanol–water solvent mixture was selected as the suitable mobile phase. Subsequently, different compositions of the mobile phase mixture [water (with 0.6% TFA)—methanol ratios: 70 : 30, 80 : 20, and 90 : 10, v/v] were tested to achieve appropriate chromatographic separation. Based on the obtained

Table 2. Statistical Analysis Values of the Calibration Curve of Proposed Method (n=6)

Features	Meloxicam
Regression equation	$y = 0.1944x - 0.0956$
Linear range (µg/mL)	1-50
Wavelength λ(nm)	290
Standard deviation of slope	50.14
Standard deviation of intercept	0.51
Correlation coefficient	0.9992

results, the mobile phase composition with water (with 0.6% TFA)—methanol ratio of 70 : 30 was determined as the optimum value and used in the study.

Method Validation

The validation of method was carried out by establishing specificity, linearity, recovery values, limits of detection (LOD), limit of quantification (LOQ), and within- and between-day precision and accuracy according to International Conference on Harmonization guidelines (ICH)[23,24] for validation of analytical procedures.

RESULTS

Specificity (Selectivity)

The method was evaluated by examining the chromatograms obtained from the standard solutions. The retention times of meloxicam and IS were determined to be 3.4 minutes and 5.9 minutes, respectively. The chromatogram depicting the increased peak area of meloxicam in relation to the concentration while keeping the internal standard constant is presented in Figure 2.

Linearity and Working Range

The linearity of the method was determined by analyzing the repeated measurements of 5 standards at each concentration within the range of 1 - 50 µg/mL. The working range was selected as the concentration range where acceptable accuracy, precision, and linearity were achieved. Calibration curves were obtained by plotting the peak area ratios (meloxicam peak area/IS peak area) against the concentration of the solution within the specified concentration range (n=6). Regression analysis of the calibration curve was performed to obtain the equation of the standard curve and the correlation coefficient.

A calibration curve was obtained by plotting the peak area ratio (meloxicam peak area/IS peak area) against the meloxicam concentration (Figure 2). The statistical analysis results of the calibration curve are presented in Table 2.

Accuracy/Precision

Three different concentrations (2, 25, and 45 µg/mL) within the calibration curves of meloxicam were prepared. The accuracy and precision values were obtained by analyzing these solutions through intraday (6 times within the same method and laboratory conditions in a single day) and interday (6 times on different days using the same method) analyses. The mean and SD of the analysis results were determined. Accuracy was expressed as relative error (RE%= (found − added)/added × 100), and precision was expressed as relative standard deviation (% RSD = SD/mean × 100) (Table 3).

Limit of Detection and Limit of Quantitaion

In the meloxicam chromatograms, the signal-to-noise (S/N) ratio was determined to be 3 for the limit of detection (LOD), and 10 for the limit of quantification (LOQ). The LOD value was determined by preparing a series of standard solutions with concentrations lower than the lowest value on the calibration curve, which is 1 µg/mL.

Analytical Recovery

Analytical recovery studies from the pharmaceutical preparation were conducted using the standard addition method. Tablet solutions were prepared as described in the "2.4. Preparation of Tablet Solutions" section. Chromatograms were obtained for the tablet solutions at a concentration of 5 µg/mL, and the peak areas were determined. Then, standard working solutions at 3 different concentrations (2, 25, and 45 mg/mL) were separately added to these tablet solutions. Chromatograms were obtained, and the peak areas were determined. The analytical recovery values were obtained by subtracting the concentration values of the added standard solutions (2, 25, and 45 mg/mL) from the total solution concentration (tablet solution + standard solution) and relating them to the concentration of the tablet solution (5 µg/mL). The average analytical recovery value was determined as 100.2% (Table 4).

DISCUSSION

Chromatography is a collection of methods widely used for the separation, identification, and determination of chemical components in mixtures, including those of unknown quantity and containing other substances. Among this group of methods, HPLC stands out as a more advantageous technique compared to others due to its accuracy, precision, repeatability, selectivity, sensitivity, recovery, ability to analyze samples in low volumes, and rapid determination of results. Thanks to these features,

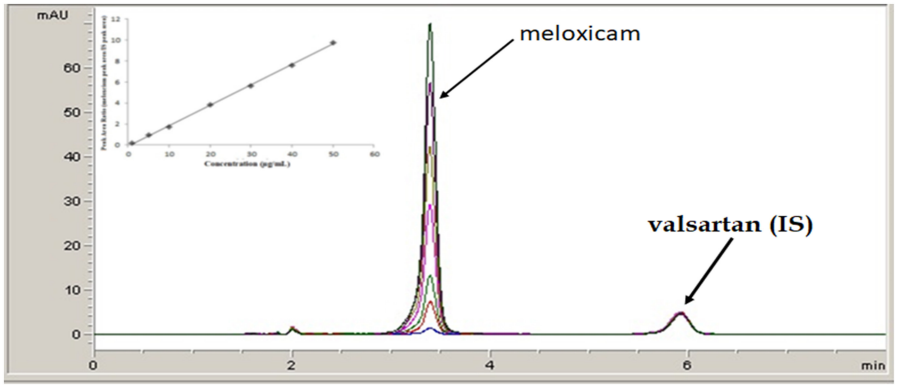

Figure 2. Calibration curve and chromatograms of meloxicam standard solutions.

Table 3. Accuracy and Precision Results of the Proposed Method

	Intra-day			Inter-day		
Added (µg/mL)	Found±SD (µg/mL)	Accuracy (RE%)	Precision (RSD%)	Found±SD (µg/mL)	Accuracy (RE%)	Precision (RSD%)
2	2.02 ± 0.07	1.0	3.5	2.05 ± 0.08	2.5	3.9
25	25.36 ± 0.62	1.4	2.4	25.17 ± 0.72	0.7	2.9
45	44.68 ± 0 .31	-0.7	0.7	44.61 ± 0.32	−0.9	0.7

RE%, relative error, RSD%, relative standard deviation; SD, standard deviation of 6 replicate determinations,

Table 4. Analytical Recovery values from tablets

Tablet	Tablet Solutions (µg/mL)	Added Standard Solutions (µg/mL)	Found±SD (µg/mL)	Analytical Recovery %	RSD %
Meksun	5	2	7.07 ± 0.10	101.4	1.41
		25	30.10 ± 0.45	102.0	1.50
		45	50.08 ± 1.01	101.6	2.02
Exen	5	2	7.04 ± 0.10	100.1	1.42
		25	30.08 ± 0.45	101.6	1.50
		45	49.94 ± 1.01	98.8	2.02
Zeloksim	5	2	6.99 ± 0.10	99.8	1.43
		25	29.96 ± 0.45	99.2	1.50
		45	50.01 ± 1.01	100.2	2.02
Melox	5	2	6.98 ± 0.10	99.6	1.43
		25	29.98 ± 0.45	99.6	1.50
		45	49.94 ± 1.01	98.8	2.02

SD, standard deviation, RSD%, relative standard deviation.

HPLC is frequently employed in the pharmaceutical industry for the quantitative analysis of pharmaceutical preparations and the analysis of drug active ingredients in biological fluids.

In HPLC studies, parameters such as temperature, column type, stationary phase, composition of the mobile phase, and the percentages of components in the mobile phase can affect the absorbance values of the analyzed substance and the analysis time. Therefore, optimization of chromatographic conditions is necessary to improve separation and obtain acceptable results. The working parameters were determined as follows: a reverse-phase C18 column (5 µm, 250 × 4.6 mm), a mobile phase consisting of 0.6% TFA—methanol (30 : 70), variable column temperature, a mobile phase flow rate of 0.8 mL/min, a wavelength of 290 nm, and an injection volume of 10 µL. When determining these parameters, existing literature data were first examined, and based on these data, certain tests were conducted to establish the most suitable ranges. As detailed in the optimization section provided in the section 'Methods,' changes in mobile phase composition, pH, and other values were made in order to achieve the highest resolution and optimal retention times for the peaks. Additionally, the aim was to propose a new method that could serve as an alternative to existing methods in the literature.

In our study, there was no need for derivatizing agents commonly used in other methods. A highly linear calibration curve was obtained without any derivatization attempts, and the recovery values indicated a satisfactory performance.

When compared to other methods in the literature, the developed method has several advantages. In comparison to the study conducted by Arayne et al[15], our method does not require the use of a buffer solution, employs a lower flow rate (0.8 mL/min in our method compared to 2 mL/min in Arayne et al[15]), and demonstrates reduced plasma interference at 290 nm compared to the commonly used wavelength of 230 nm. Mahmood et al[19] employed a 0.2 N buffer and completed the analysis in 7.5 minutes. In contrast, our developed method has a shorter analysis time and does not require the use of a buffer solution, which can negatively affect column lifetime. Joseph-Charles and Betucat[25] performed their analysis with a flow rate of 1.5 mL/min and a 0.05M Tris and 0.05 M acetate buffer, while our method is more economical and less damaging to the column and other HPLC equipment due to the buffer-free application. In the study by Viqnaduzzo et al[17], the active ingredient meloxicam was analyzed at 225 nm using a phosphate buffer component at pH 5.9. Bandarkar et al[18], in their study on pharmaceutical preparations, reported a linear range of 4-20 µg/mL, while our method stands out as a more sensitive approach with a linear range of 1-50 µg/mL. Additionally, our method has a shorter analysis time compared to this method. Overall, the developed method offers several advantages compared to other methods in terms of reduced interference, improved efficiency, and shorter analysis time.

A new HPLC method was developed as an alternative to the existing methods in the literature for the quantitative analysis of the active ingredient meloxicam in standard solutions and pharmaceutical preparations. The validity tests demonstrated that this method is sensitive, selective, accurate, precise, and reproducible for meloxicam, thus indicating its applicability for the quantitative analysis of meloxicam in pharmaceutical preparations. The data obtained from this study are believed to provide guidance for future research endeavors.

Ethics Committee Approval: Since this study is an in vitro (quantification in pharmaceutical preparations) study, ethics committee approval is not required.

Informed Consent: This study is not about the patient.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.K., R.S.C.; Design – Y.K., R.S.C.; Supervision– Y.K., R.S.C.; Resources – Y.K.; Materials – Y.K.; Data Collection and/or Processing – Y.K.; Analysis and/or Interpretation – B.B.; Literature Search – B.B.; Writing Manuscript – Y.K., B.B.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

REFERENCES

1. Nageswara Rao R, Meena S, Raghuram Rao A. An overview of the recent developments in analytical methodologies for determination of COX-2 inhibitors in bulk drugs, pharmaceuticals and biological matrices. *J Pharm Biomed Anal*. 2005;39(3-4):349-363. [CrossRef]
2. Del Tacca M, Colucci R, Fornai M, Blandizzi C. Efficacy and tolerability of meloxicam, a COX-2 preferential nonsteroidal anti-inflammatory drug – a review. *Clin Drug Investig*. 2002;22(12):799-818. [CrossRef]
3. Cahoon EK, Rajaraman P, Alexander BH, Doody MM, Linet MS, Freedman DM. Use of nonsteroidal anti-inflammatory drugs and risk of basal cell carcinoma in the United States radiologic technologists study. *Int J Cancer*. 2012;130(12):2939-2948. [CrossRef]
4. Meloxicam properties. Available at: https://www.worldofchemicals.com/chemicals/chemical-properties/meloxicam.html.
5. Leal LB, Bedor DCG, Melo EKS, Oliveira EJ, Santana DP. Determination of meloxicam in human plasma administrated with four drugs

by LC method: application to a pilot bioavailability study. *Lat Am J Pharm*. 2011;30(10):1883-1888.
6. Argoff CE. Recent developments in the treatment of osteoarthritis with NSAIDs. *Curr Med Res Opin*. 2011;27(7):1315-1327. [CrossRef]
7. Awasthi SS, Kumar TG, Manisha P, Preeti Y, Kumar SS. Development of Meloxıcam formulatıons utılızıng ternary complexatıon for solubılıty enhancement. *Pak J Pharm Sci*. 2011;24(4):533-538.
8. Nikolaychuk PA. UV-spectrophotometric determination of the active pharmaceutical ingredients meloxicam and nimesulide in cleaning validation samples with sodium carbonate. *Multidiscip Sci J*. 2023;6(2):248-266. [CrossRef]
9. Mahood AM, Najm NH. Spectrophotometric Estamation of meloxicam using charge transfer complex. *IOP Conf Ser Mater Sci Eng*. 2019;571(1). [CrossRef]
10. Hasan SH, Othman NS, Surchi KM. Development and validation of a UV spectrophotometric method for determination of meloxicam in bulk and in tablet formulations. *Int J Pharm Sci*. 2015;6(7):1040-1045.
11. Gurupadayya BM, Trinath MN, Shilpa K. Spectrophotometric determination of meloxicam by sodium nitroprusside and 1,10-phenanthroline reagents in bulk and its pharmaceutical formulation. *Indian J Chem Tech*. 2013;20:111-115.
12. Tomuta I, Iovanov R, Bodoki E, Leucuta SE. Quantification on intact tablets by near infrared spectrometry and Chemometry. *Farmacia*. 2010;58(5):559-571.
13. Nemutlu E, Kir S. Method development and validation for the analysis of meloxicam in tablets by CZE. *J Pharm Biomed Anal*. 2003;31(2):393-400. [CrossRef].
14. Sahoo NK, Sahu M, Rao PS, Rani NS, Devi JI, Ghosh G. Validation of assay indicating method development of meloxicam in bulk and some of its tablet dosage forms by RP-HPLC. *Springerplus*. 2014;3:95. [CrossRef]
15. Arayne MS, Sultana N, Siddiqui FA. A new RP-HPLC method for analysis of meloxicam in tablets. *Pak J Pharm Sci*. 2005;18(1):58-62.
16. Nemutlu E, Sayın F, Başcı NE, Kır S. A validated HPLC method for the determination of meloxicam in pharmaceutical preparations. *Hacettepe Univ J Fac Pharm*. 2007;27:107-118.
17. Vignaduzzo SE, Castellano PM, Kaufman TS. Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations. *J Pharm Biomed Anal*. 2008;46(2):219-225. [CrossRef]
18. Bandarkar FS, Vavia PR. A Stability indicating HPLC method for the determination of meloxicam in bulk and commercial formulations. *Trop J Pharm Res*. 2009;8(3):257-264. [CrossRef]
19. Mahmood KT, Khan B, Ashraf M, Haq IU. Specific and simple HPLC assay of ecofriendly meloxicam in pharmaceutical formulations. *J Pharm Sci Res*. 2010;2(12):878-883.
20. Ahmad R, Hailat M, Zakaraya Z, Al Meanazel O, Abu Dayyih W. Development and validation of an HPLC method for the determination of meloxicam and pantoprazole in a combined formulation. *Analytica*. 2022;3(2):161-177. [CrossRef]
21. Parys W, Bober K, Pyka-Pająk A, Dołowy M. The application of TLC and densitometry for quantitative determination of meloxicam in tablets. *Curr Pharm Anal*. 2019;15(7):785-794. [CrossRef]
22. Desai N, Amin P. Stability indicating HPTLC determination of meloxicam. *Indian J Pharm Sci*. 2008;70(5):644-647. [CrossRef].
23. ICH. Q2B guideline validation of analytical procedures: methodology. International Conference on Harmonization of Technical Requirements for the Registration of Drugs for Human Use. Geneva, Switzerland; 1997.
24. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Geneva; 2005:1-13.
25. Joseph-Charles J, Bertucat M. Determination of meloxicam in tablet formulations by ultraviolet spectrophotometry and high-performance liquid chromatography. *Ana. Lett.* 1999; 32(10): 2051-2059.

ATATURK UNIVERSITY PUBLICATIONS

Review

DOI: 10.5152/Pharmata.2023.1321447

Obesity and Obesity-Related Diseases and Treatments

Amine Sena AYDIN[1]
Tuba Rümeysa SİVRİ[2]
Halise İnci GÜL[3]

[1]Department of Pharmaceutical Chemistry, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey
[2]Atatürk University, Faculty of Pharmacy, Erzurum, Turkey
[3]Department of Pharmaceutical Chemistry, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey

ABSTRACT

Obesity is a disease that impairs body health as a result of abnormal or excessive fat deposit in the adipose tissue. Obesity and overweight are caused when the amount of energy taken during nutrition is higher than the amount spent during metabolism and physiological activities. Obesity is a chronic and common disease with increasing prevalence worldwide affecting both adults and children. Obesity is a multifactorial disease affected by many environmental and genetic factors and is known to be an important risk factor for early mortality, metabolic, and cardiovascular complications. The fact that obesity treatment is a long and difficult process and its cost is high emphasizes the importance of preventing obesity. For this purpose, obesity prevention studies including diet education, physical activity, and behavioral changes should be initiated and carried out. When preventive measures are insufficient, different treatment options come to the fore including diet, exercise, lifestyle changes, medication, and surgery. In this article, data on the definition of obesity, its causes, and treatment options have been compiled in order to determine the ideal methods for the prevention and treatment of obesity.

Keywords: Anti-obesity agents, drug, obesity, obesity treatment

INTRODUCTION

Obesity is defined as a chronic metabolic disease described by increased fat stores in the body.[1] According to the World Health Organization (WHO) valuation, those with a body mass index between 25 and 29.9, which is calculated by dividing the body weight by the square of the height, are considered "overweight" and those above 30 are considered "obese."[2] The proportion of body fat in people with ideal weight should be 12%-18% in men and 20%-30% in women. If the body fat ratio is more than 22%-25% in men and 32%-35% in women, the presence of obesity is mentioned.[3]

It is known that the obesity occurs as a result of the interaction of genetic, psychological, physical, environmental, and socioeconomic factors.[1,4] There are risk factors such as age, hormonal and metabolic factors, gender, sociocultural factors, income status and education level, genetic factors, sedentary lifestyle, excessive and wrong nutrition, some medications used, frequent dieting, marital status, smoking and alcohol use, and irregular sleep in the formation of obesity.[5,6] The first phase of obesity treatment is to aim an achievable body weight loss for patients. In this way, it is aimed to provide the individual with adequate and correct nutrition habits, to increase the quality of life of the individual, and to reduce the risks of obesity-related diseases and deaths in the individual.[7]

Diagnosis, Etiology of Obesity, and Affecting Factors

The body mass index (BMI) is widely used to determine obesity according to the obesity classification made by the World Health Organization. Body mass index is known as a value acquired by dividing the body weight (kg) of the individual by the square of the height (meter) (BMI = kg/m^2). Since BMI is a cheap, simple, and reliable method, it is frequently used as an obesity diagnosis method (Table 1).[8]

Obesity is a multifactorial disease and occurs as a result of the interaction of genetic, physical, psychological, environmental, and socioeconomic factors.[9] Hormonal disorders such as hypothyroidism cause an increase in fat accumulation due to a decrease in basal metabolic rate. Insulinoma, Cushing's syndrome, hypothyroidism, polycystic ovary, hypogonadism, and Binge eating disorder can cause obesity.[10] Some drug classes used in the treatment of various diseases such as antidepressants, antiepileptics, and neuroleptics can also cause weight gain as a side effect. The most important findings supporting the role of heredity in obesity have been obtained from studies with BMI in identical twins, which is a multifactorial disease.[11] The fact that the concordance found in monozygotic twins is higher than in dizygotic twins in these studies indicates the effect of heredity in obesity.[12,13] Obesity is affected by environmental factors as well as heredity. For example, it is suggested that the desire of obese patients to overeat is a habit acquired from the family environment. In addition, excessive television

Received: 23.07.2023
Accepted: 03.07.2023
Publication Date: 31.07.2023

Corresponding Author:
Amine Sena AYDIN
E-mail: sena.aydin@atauni.edu.tr

Cite this article as: Aydın AS, Sivri TR, Gül Hİ. Obesity and obesity-related diseases and treatments. *Pharmata* 2023;3(3):64-70.

Table 1. The Ranges of Body Mass Index Values

	BMI (kg/m²)
Severe thinness	<16.0
Moderate thinness	16.0-16.9
Mild thinness	17.0-18.4
Normal range	18.5-24.9
Overweight (pre-obese)	25.0-29.9
Moderately obese	30.0-34.9
Severely obese	35.0-39.9
Very severely obese	≥40.0

watching, long-term computer use, and lack of physical activity are known as habits originating from family life.[14]

Prevalence of Obesity

Obesity, which is a health problem that has increased so much in the world, is now described as a pandemic.[15] According to the WHO data, while the probability of obesity in the 5-19 age group of children and adolescents was less than 1% in 1975, 8% of boys and 6% of girls were in the obese group in 2016.[15] According to the WHO European Region Obesity Report 2022 published by the WHO, more than 59% of the people (63% of men and 54% of women) in the European Region are considered overweight or obese. Approximately, 1 in 3 children (29% in boys, 27% in girls) is overweight or obese; it is stated that 1 out of every 10 children is obese.[16]

It is known that the prevalence of obesity in the adult population in Turkey exceeds 30%. Although the prevalence of obesity is higher in women than in men, there has been a rapid increase in male obesity recently.[17] According to the results of the studies conducted in 2016, it is stated that Turkey is one of the countries where obesity is more common with a prevalence value of 29.5% among the WHO European Region countries.[18] According to WHO's European Region Obesity Report 2022 data, Turkey is stated as the country with the highest obesity prevalence in the European Region. In Turkey, 66.8% of the adult population are considered overweight and 32.1% are obese.[19] In the Turkey Childhood Obesity Research Initiative Study (COSI-TUR 2016), it was determined that 9.9% of children in the 7-8 age group in Turkey were obese and 14.6% were overweight . In this study, it was determined that 1 out of every 4 children in the 7-8 age group in Turkey is obese or overweight.[17,20]

Health Problems Caused by Obesity

Obesity has negative effects on the cardiovascular system, endocrine system, gastrointestinal system, skin, musculoskeletal system, genitourinary system, respiratory system, and psychosocial status.[21] Insulin resistance, type 2 diabetes, hypertension, hyperlipidemia, gallbladder diseases, coronary artery disease, some types of cancer, osteoarthritis, paralysis, sleep apnea, asthma, pregnancy complications, fatty liver, menstrual irregularities, pre- and post-operative complications, musculoskeletal system problems, mental problems, anorexia nervosa, overeating, excessive hair growth, social maladjustment, skin infections due to subcutaneous fat tissue, fungal infections in the groin and feet are important health problems caused by obesity.[22] The effects of obesity on body systems are detailed in Table 2.

Treatment of Obesity

The aim of obesity treatment is to increase the quality of life of the person and to provide an adequate and balanced nutrition habit by providing a sufficient loss of body weight and by minimizing the risk of obesity-related morbidity and mortality.[23] Obesity treatment methods can be listed as medical nutrition (diet) therapy, exercise therapy, behavior modification therapy, pharmacological therapy, and surgical therapy.[24]

Medical Nutrition (Diet) Therapy

In the medical nutrition treatment of obesity, the aim is to reach the ideal weight and keep the person's weight at the ideal level by creating an energy deficit and reducing the body fat stores without loss of muscle and mass in the muscles and the vital organs.[25] According to the energy needs of the people, dietitians recommend low-calorie diets that give an average of 1200–1600 kcal/day for men and 1000-1200 kcal/day for women.[26] Diet programs should be prepared individually depending on the person's eating habits and food consumption.[27]

Exercise Therapy

Regular physical activities have an important role in regulating the energy balance, reducing the health problems caused by obesity and the mortality rate due to these health problems. Before starting a new physical activity program for obese individuals, cardiopulmonary checks should be made, chronic diseases and symptoms should be evaluated, and personalized exercise programs should be selected.[28] According to the Public Health Institution of Turkey, an individual should do physical activity for at least 5 days a week, 40-60 minutes once a day, or 20-30 minutes twice a day. Oxygen consumption should also be between 50% and 70% during exercise.[29]

Behavior Modification Therapy

One of the most important methods of obesity treatment is behavioral therapy. The aim of the treatment of obesity with behavior modification therapy is to create awareness and change in the patient's eating and activity habits and to gain positive behaviors related to physical activity and nutrition that cause excessive weight gain, lifestyle and habits. The first condition of achieving permanent weight control is to motivate the patient

Table 2. The Effects of Obesity on Body Systems

Cardiovascular diseases	Hypertension, coronary heart disease, deterioration in lipid profile, stroke, venous thrombosis, pulmonary embolism, varicose veins
Cancers	Urogenital (endometrium, cervix, ovary, prostate, kidney), gastrointestinal (colorectal, liver, gallbladder, esophagus, small intestine, pancreas) system tumors and leukemia, multiple myeloma, lymphoma, and breast cancer
Metabolic	Insulin resistance and type-2 diabetes mellitus, metabolic syndrome, gallstones, hyperuricemia, and gout
Hormonal	Polycystic ovary syndrome, menstrual cycle irregularities, hyperandrogenism, decreased levels of sex hormones (estrogen, testosterone), decrease in growth hormone and prolactin response, infertility, increase in cortisol synthesis, acanthosis nigricans
Rheumatological	Osteoarthritis (especially lower extremity joints), entrapment neuropathies, immobility, low back pain
Pulmonary	Asthma, decrease in total lung and functional residual capacity, increase in diffusion capacity and residual volume, sleep apnea syndrome, obesity hypoventilation syndrome (Pickwickian syndrome)
Gastrointestinal	Gallstone (cholelithiasis), fatty liver disease, reflux, esophageal hernia
Urinary	Incontinence, glomerulopathy, proteinuria, nephrotic syndrome
Psychological	Decreased self-confidence, depression, dementia, anxiety disorders, stigmatization, social exclusion and unemployment, deterioration in body image
Others	Idiopathic intracranial hypertension, skin infections (cellulitis, carbuncle, etc.), stasis, and lymphedema in the legs

Table 3. Pharmacological Drugs that Can be Used in the Treatment of Obesity

Medicines that reduce fat absorption	Orlistat
Antidiabetic drugs	Metformin, exenatide, liraglutide, pramlintide
Sympathomimetic drugs	Phentermine
Selective serotonin receptor agonists	Lorcaserin
Antiepileptic drugs	Topiramate
Combination treatments	Topiramate-phentermine, Bupropion SR-Naltrexone

to achieve the goal.[30] For a more active life, patients should be offered simple suggestions such as not snacking while watching TV, getting off the bus or minibus one stop before arriving at their destination, using the stairs instead of the elevator, and parking their vehicles at the furthest distance in places such as shopping malls. In addition, patients should be asked to monitor their weight and food intake throughout the treatment. Since frequent control and effective and long-term social support will increase the chances of success, measures should be taken to regulate the social environment of the patients.[31]

Surgical Treatment

Today, surgical treatment methods are used safely and effectively in the treatment of obesity. Surgical treatment is applied to patients who do not respond to other obesity treatment methods, have a BMI >40 kg/m^2 or a BMI in the range of 35-40 kg/m^2, and are at high risk for obesity-related diseases. In the surgical treatment method, intestinal bypass, laparoscopic gastric band application, partial biliopancreatic bypass, gastroplasty, adjustable silicone gastric band insertion, and gastric balloon application are some of the applications used.[32]

Pharmacological Therapy

Pharmacological treatment in obesity is applied if the patient has a BMI of 27-30 kg/m^2 and at least one of the obesity-related risk factors or complications. Pharmacological treatment is also applied if there is no response to behavioral change treatment that includes healthy nutrition and exercise.[33] The drugs to be used in the treatment of obesity should prevent the storage of fat in the body or to increase the use of fat to dissolve the fat stores. One of the important factors that will reduce the accumulation of fat in the body is the limitation of calorie intake, and according to this method, it is necessary to control the appetite.[34]

Another important method to reduce the fat percentage is to limit the amount of fat that passes into the systemic circulation. Methods increasing thermogenesis and basal metabolism can reduce fat stores by elevating energy consumption in the body. Such methods generally act through the activation of the sympathetic system.[35] The features that should be present in an ideal obesity drug are as follows: it should cause dose-related weight loss; it should ensure the continuity of the reached target weight; it should be safe when it is used chronically; it should not develop tolerance; and finally it should not cause abuse or addiction.[36]

Drugs Used in the Obesity Treatment

Pharmacological treatment of obesity is applied if the patient's BMI is between 27 kg/m^2 and 30 kg/m^2 and at least one of the obesity-related risk factors or complications is present. In addition, pharmacological treatment is applied in case of failure to respond to behavioral therapy including healthy nutrition and exercise.[37] Since there is excessive fat storage in the body in obese patients, the used in the treatment of obesity should prevent the storage of fat or increase the use of fat to melt the stores. One of the most important factors that will reduce the accumulation of fat in the body is the restriction of calorie intake, that is, food intake, and in this method, it is aimed to control the appetite.[37] The success of the drug in the treatment of obesity is measured by the degree of weight loss and the reduction in associated risk factors. Weight loss of 2 kg in the first month of drug therapy, losing more than 5% of the initial body weight within 3-6 months, and maintaining this target weight indicate that the treatment is successful.[38] Pharmacological drugs that can be used in the treatment of obesity are classified as shown in Table 3.[39,40] The mechanisms of action, approval bodies, and side effects of drug-active ingredients used in the treatment of obesity are given in Table 4.[41-59] The chemical structures of some commonly used anti-obesity drugs are given in Figure 1.

Orlistat

Orlistat, can be preferred in obese people with BMI ≥ 30 kg/m^2 or can also be chosen as one of the drugs used in the treatment of excess body weight in patients with ≥28 kg/m^2.[41] Orlistat acts as a specific, potent, irreversible food lipase inhibitor, which reduces the calorie content of meals consumed by reducing the

Table 4. Generally Used Anti-Obesity Drugs

Drug	Approving Bodies	Mechanism	Adverse Effects	References
Orlistat	FDA - 1999 EMA -1998	Pancreatic lipase inhibitor	Decreased absorption of fat-soluble vitamins, oily stools, fecal urgency, bloating, gas, abdominal pain, and diarrhea	41-43
Topiramate	FDA- 2012	Anti-convulsant agent	Gastrointestinal adverse effects, increased nervousness, sweating, tremors, hypersomnia, insomnia, and fatigue	44,45
Phentermine	FDA- 2012	Sympathomimetic amine, appetite suppressant	Increased blood pressure and pulse rate, constipation, insomnia, dizziness, tremor, headache, and palpitation	45-47
Liraglutide	FDA- 2014 EMA- 2015	GLP-1 agonist	Nausea, medullary thyroid tumor in rats and mice, pancreatitis, headache, constipation, and heartburn	48,49
Semaglutide	FDA- 2017	GLP-1 agonist		49,50
Dulaglutide	FDA- 2014 EMA- 2014	GLP-1 agonist		51,52
Tirzepatide	EMA- 2022	GLP-1 agonist	Nausea, diarrhea, vomiting, dyspepsia, constipation, abdominal pain, dizziness, and hypoglycemia	56
Lorcaserin	FDA- 2012	Selective 5HT-2C receptor agonist	Depression, infection, headache, nausea, and dizziness	53-55
Bupropion and naltrexone	FDA- 2014 EMA- 2015	Naltrexone:OpioidreceptorantagonistBupropion:Norepinephrine/dopamine reuptake inhibitör	Nausea, headache, insomnia, constipation, dizziness, and vomiting	57-60

FDA, Food and Drug Administration; EMA, European Medicines Agency

absorption of fats in the gastrointestinal tract.[42] Covalent binding of gastric and pancreatic lipases with the active serine site in the gastrointestinal tract lumen prevents pancreatic and gastric enzymes from hydrolyzing dietary fat into absorbable free fatty acids and monoglycerols.

The pharmacological action of orlistat is dose dependent, with the optimal therapeutic dose recommended as 180-360 mg/day 1 hour after each main meal or divided into 3 doses up to 1 hour. In case of skipping a meal, orlistat is not required. Patients should follow a nutritious and balanced diet with a fat content of no more than 30%. It is also recommended to distribute fat intake evenly at each meal to minimize gastrointestinal side effects. If the patient's meal does not contain fat, he may skip the orlistat dose. Caution is required when it is used with cyclosporine. In cases where orlistat is used orally, it is excreted in the stool for 3-5 days. Orlistat is contraindicated in patients with pregnancy and lactation, chronic malabsorption syndrome, or cholestasis.[43] Side effects such as oily spotting, leakage with gas, sudden need to defecate, loose or watery stools, oily stools, increased frequency of defecation, and stool incontinence can be seen with the use of orlistat. Side effects increase as the fat ratio in the diet increases and the absorption of fat-soluble vitamins is impaired. Therefore, when using orlistat, a fat-soluble vitamin supplement is recommended.[42]

Topiramate

Topiramate [2,3:4,5-Bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate] acts as a glutamate antagonist, carbonic anhydrase inhibitor, and gamma-aminobutyric acid (GABA) agonist. It is an antiepileptic drug and causes weight loss as a side effect in epilepsy patients. The exact reason why it causes weight loss is not known.[44,45]

Phentermine

Phentermine (2-methyl-1-phenylpropan-2-amine) acts as a central sympathomimetic leading to increased secretion of norepinephrine, dopamine, and serotonin. It was discontinued in 1997.[45,46] Phentermine is an appetite suppressant belonging to the amphetamine and phenethylamine class. It is known that short-term use of appetite suppressants, together with exercise, diet, and behavior changes, provides weight loss. Phentermine is generally used in obese individuals with serious weight problems. It has the potential to be addictive because its structure is similar to amphetamines.[47]

Liraglutide

Liraglutide can be given subcutaneously once daily in patients with type 2 diabetes mellitus (T2DM) at a target dose of 1.8 mg as a short-acting glucagon-like peptide-1 (GLP-1) analog. In patients with prediabetes, T2DM, dyslipidemia and OSAS (obstructive sleep apnea syndrome) and abnormal body weight, BMI ≥30 kg/m^2 or 27 kg/m^2 to <30 kg/m^2, the indication has been expanded to a target dose of 3 mg once daily since 2014 in the USA and 2015 in Europe.[48] Liraglutide, when used at a dose of 3 mg daily for 32 weeks, has been reported to contribute not only to significant weight loss but also to the androgen index and increase in menstrual frequency or regulation of menstrual frequency.[49]

Semaglutide

Semaglutide, which is a long-acting GLP-1 analog approved by the FDA and EMEA, is used in patients with a BMI of ≥30 kg/m^2 or ≥27 kg/m^2 and at least one weight-related condition.[49] Studies have shown that the use of 2.5 mg semaglutide for 68 weeks resulted in a >20% reduction in body weight in 13.1%-36.6% of patients, and a 1.0 mg dose causes weight reduction in 4.7% of patients. Orally administered semaglutide has proven to have a

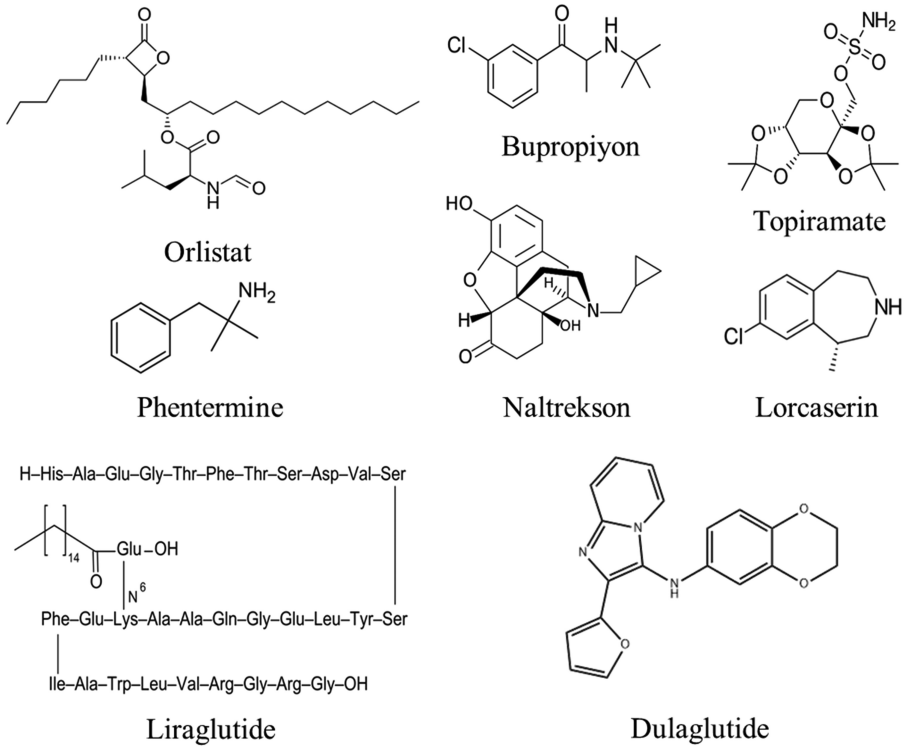

Figure 1. The chemical structures of some commonly used anti-obesity drugs.

significantly beneficial effect in reducing hunger, body fat, and body weight in patients with T2DM.[50]

Dulaglutide

Dulaglutide is used as a GLP-1 agonist in the treatment of T2DM in addition to its weight-reducing effect. In addition to insulin therapy, it has been reported to significantly reduce body weight.[51] It has been reported that patients with a diagnosis of eating disorders showed a reduction in the number of eating episodes after 12 weeks of treatment, and a significant reduction in body weight, glycated hemoglobin levels, and BMI during dulaglutide treatment compared to placebo.[52]

Lorcaserin

Lorcaserin acts as a selective agonist of the [(5R)-7-chloro-5-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine] 5-HT serotonin receptor.[53] FDA approves this drug for the purpose of promoting weight loss in obese patients with or without T2DM in long-term use with lifestyle modification. However, this drug cannot be approved due to EMEA's safety concerns. Studies show that lorcaserin reduces the incidence of DM by 19% in pre-diabetic patients and by 23% in patients without DM.[38] In diabetic patients, a 21% reduction in diabetic microvascular complications has been observed and it is known that there is an improvement in quality of life independent of dose.[54] It is emphasized that lorcaserin is effective both in the first year and in the second year of treatment. According to the FDA, lorcaserin should be discontinued if at least 12% of weight loss is not achieved within the first 5 weeks.[55]

Tirzepatide

Tirzepatide is a new drug that has been approved by the FDA in 2022 for the treatment of obesity and acts as a double GLP-1 and Gastric Inhibitory Polypeptide (GIP) receptor agonist. Subcutaneous application once a week is sufficient because it has a half-life of about 5 days. According to studies, when semaglutide and tirzepatide are compared, it is seen that tirzepatide has a much higher efficacy in weight loss.[56]

Bupropion and Naltrexone Combination

In patients with BMI ≥ 30 kg/m^2 or ≥27 kg/m^2 and T2DM, bupropion in combination with naltrexone can be used as another therapeutic option for patients with at least one co-morbidity such as dyslipidemia.[57] According to a study, it has been proven that in obese patients with BED (patient with binge eating disorder), using a long-acting preparation of the combination of naltrexone and bupropion not only significantly reduces body weight but also improves pathological eating behavior.[58] This drug combination is not to be used in patients with a history of seizures, eating disorders, or patients using alcohol. It should not be used with central nervous system depressants, especially opioids.[60]

DISCUSSION

Obesity is becoming a growing problem in children and adults around the world. According to studies on obesity, besides factors such as genetic structure, age, gender, dietary habits, sedentary lifestyle, socioeconomic and cultural level, media and screen exposure are the most important factors causing obesity. In this context, it is recommended to plan nutrition education to be given to both parents and children. Type 2 diabetes mellitus and obesity are among the well-established diseases that do not have a specific treatment so far but can be kept under control by including the appropriate treatment as well as lifestyle changes. The alarming increase in obese patient numbers worldwide requires new scientific advances to streamline administration, reduce dosing frequency, and address multiple issues in a single drug.

The fact that the drugs used in the treatment of obesity have undesirable side effects, the effects are within certain limits, and the patient regains weight when the drug is discontinued limits the long-term use of the drugs in the market. When obesity drugs are used in the treatment, the benefit/harm ratio is taken into account in the treatment with these drugs, since they have more side effects than diet and exercise. In this article, we have compiled data on obesity treatment methods and antiobesity drugs used in treatment. As the knowledge about the causes of obesity and the diseases it causes increases, it will be easier to discover more active pharmaceutical ingredients for medical treatment and to develop other treatment methods. Pharmacotherapy modalities of diabetes and obesity are developing rapidly around the world, and an increasing number of drug targets have been identified. Although these drugs are associated with clinically significant weight loss, clinical trials are ongoing. It is anticipated that more drugs will be approved for the treatment of obesity as clinical trials of candidate and existing drugs continue. These methods are very promising as they will reduce the tendency to prefer more risky methods such as bariatric surgery.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.S.A., H.İ.G.; Design – A.S.A., H.İ.G.; Supervision – H.İ.G.; Resources – A.S.A.; Data Collection and/or Processing – A.S.A.; Literature Search – T.R.S.; Writing Manuscript – A.S.A., T.R.S.; Critical Review – H.İ.G.

Acknowledgments: Amine Sena AYDIN would like to thank the Turkish Scientific and Technical Research Council (TUBITAK) for the scholarship for his postgraduate program.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

REFERENCES

1. Ünlü TN, Deniz D. Türkiye'de uygulanan obezite politikaları; Almanya uygulama karşılaştırılması. *Selçuk Sağlık Derg*. 2022;3(1):62-78.
2. Yüksel E, Adiyaman F, Keçeli D, ve ark. Obezite konusu basında nasıl çerçevelenmektedir? *Selçuk İletişim*. 2014;8(2):149-176.
3. Balli E. (2013). *Obezite, obezitenin tetiklediği hastalıklar ve tedavileri*. TC Erciyes Üniversitesi, Eczacılık Fakültesi Farmasötik Kimya Anabilim Dalı, Kayseri.
4. Mutch DM, Clément K. Genetics of human obesity. *Best Pract Res Clin Endocrinol Metab*. 2006;20(4):647-664. [CrossRef]
5. Malik VS, Willett WC, Hu FB. Global obesity: trends, risk factors and policy implications. *Nat Rev Endocrinol*. 2013;9(1):13-27. [CrossRef]
6. Danaei G, Ding EL, Mozaffarian D, et al. The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. *PLOS Med*. 2009;6(4):e1000058. [CrossRef]
7. Steinbeck KS, Lister NB, Gow ML, Baur LA. Treatment of adolescent obesity. *Nat Rev Endocrinol*. 2018;14(6):331-344. [CrossRef]

8. Kose O, Canakci V, Arabaci T, Saglam E. Obesity and periodontitis. *Clin Exp Health Sci*. 2012;2(2):89.
9. Safaei M, Sundararajan EA, Driss M, Boulila W, Shapi'i A. A systematic literature review on obesity: understanding the causes & consequences of obesity and reviewing various machine learning approaches used to predict obesity. *Comput Biol Med*. 2021;136:104754. [CrossRef]
10. Reinehr T. Obesity and thyroid function. *Mol Cell Endocrinol*. 2010;316(2):165-171. [CrossRef]
11. Bays HE, González-Campoy JM, Bray GA, et al. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Rev Cardiovasc Ther*. 2008;6(3):343-368. [CrossRef]
12. Farooqi IS. Genetic and hereditary aspects of childhood obesity. *Best Pract Res Clin Endocrinol Metab*. 2005;19(3):359-374. [CrossRef]
13. Clement K, Boutin P, Froguel P. Genetics of obesity. *Am J Pharmacogenomics*. 2002;2(3):177-187. [CrossRef]
14. Turconi G, Guarcello M, Maccarini L, Bazzano R, Zaccardo A, Roggi C. BMI values and other anthropometric and functional measurements as predictors of obesity in a selected group of adolescents. *Eur J Nutr*. 2006;45(3):136-143. [CrossRef]
15. Mohajan D, Mohajan HK. Obesity and its related diseases: a new escalating alarming in global health. *J Innov Med Res*. 2023;2(3):12-23. [CrossRef]
16. Oppert JM, Ciangura C, Bellicha A. Physical activity and exercise for weight loss and maintenance in people living with obesity. *Rev Endocr Metab Disord*. 2023:1-13. [CrossRef]
17. Erem C. Prevalence of overweight and obesity in Turkey. *IJC Metab Endocr*. 2015;8:38-41. [CrossRef]
18. Kim KB, Shin YA. Males with obesity and overweight. *J Obes Metab Syndr*. 2020;29(1):18-25. [CrossRef]
19. WHO. *WHO European Regional Obesity Report 2022*. Geneva: World Health Organization. Regional Office for Europe; 2022.
20. Düzgun-Öncel B, Karaoğlan D. *Determinants of childhood obesity: evidence from Turkey*. In Academic Studies, 2019:193.
21. James WPT, Jackson-Leach R, Mhurchu CN, et al. Overweight and obesity (high body mass index). *Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors*. 2004;1:497-596.
22. Kyrou I, Randeva HS, Tsigos C, Kaltsas G, Weickert, MO. Clinical problems caused by obesity. In *Endotext,* eds KR, Feingold, KR, Anawalt, B, Boyce A, et al. South Dartmouth, MA: MDText.com, Inc, 2018.
23. Karataş S, Obez HN, Obeziteye Yönelik OKB. Tutumları ve yaşam kalitelerinin karşılaştırılması: Niteliksel çalışma. *Acıbadem Univ Sağlık Bilimleri Derg*. 2020;4:589-595.
24. Blüher M, Aras M, Aronne LJ, et al. New insights into the treatment of obesity. *Diabetes Obes Metab*. 2023;25(8):2058-2072. [CrossRef]
25. Alexander L, Christensen SM, Richardson L, et al. Nutrition and physical activity: an obesity medicine association (OMA) clinical practice statement 2022. *Obes Pillars*. 2022;1:100005. [CrossRef]
26. Wiechert M, Holzapfel C. Nutrition concepts for the treatment of obesity in adults. *Nutrients*. 2021;14(1):169; Recent Advances and Remaining Challenges. 2021:1.
27. Hensrud DD. Diet and obesity. *Curr Opin Gastroenterol*. 2004;20(2):119-124. [CrossRef]
28. Takahashi T, Kohzuki M. Effect of weight loss and exercise therapy on obesity-related respiratory disorders. *Asian J Hum Serv*. 2019;17:95-106. [CrossRef]
29. Kahraman MS, Güriz SO, Özdel K. Yetişkinlerde obezite: Biyolojik ve psikolojik tedaviler için genel bir gözden geçirme. *Klin Psikiyatr Derg*. 2014;17(1).28-40.
30. Burke LE, Wang J, Sevick MA. Self-monitoring in weight loss: a systematic review of the literature. *J Am Diet Assoc*. 2011;111(1):92-102. [CrossRef]
31. Wadden TA, Tronieri JS, Butryn ML. Lifestyle modification approaches for the treatment of obesity in adults. *Am Psychol*. 2020;75(2):235-251. [CrossRef]
32. Haywood C, Sumithran P. Treatment of obesity in older persons—a systematic review. *Obes Rev*. 2019;20(4):588-598. [CrossRef]
33. Berberoğlu Z, Hocaoglu C, Sorunu KS. Küresel sağlık sorunu 'obezite': Güncel bir gözden geçirme. *Celal Bayar Univ Sağlık Bilimleri Enstitüsü Derg*. 2021;8(3):543-552. [CrossRef]
34. Montan PD, Sourlas A, Olivero J, Silverio D, Guzman E, Kosmas CE. Pharmacologic therapy of obesity: mechanisms of action and cardiometabolic effects. *Ann Transl Med*. 2019;7(16):393. [CrossRef]
35. Jackson VM, Breen DM, Fortin JP, et al. Latest approaches for the treatment of obesity. *Expert Opin Drug Discov*. 2015;10(8):825-839. [CrossRef]
36. Kaplan LM. Pharmacologic therapies for obesity. *Gastroenterology Clinics of North America*. 2010;39(1):69-79. [CrossRef]
37. Kayar H, Semra U. Çağımızın hastalığı obezite ve tedavisi. *Mersin Univ Sağlık Bilimleri Derg*. 2013;6(2):1-8.
38. Williams DM, Nawaz A, Evans M. Drug therapy in obesity: a review of current and emerging treatments. *Diabetes Ther*. 2020;11(6):1199-1216. [CrossRef]
39. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. *Obes Res*. 2001;9(suppl 4):228S-233S. [CrossRef]
40. Harvey EL, Glenny AM, Kirk SF, Summerbell CD. An updated systematic review of interventions to improve health professionals' management of obesity. *Obes Rev*. 2002;3(1):45-55. [CrossRef]
41. Sumithran P, Proietto J. Benefit-risk assessment of orlistat in the treatment of obesity. *Drug Saf*. 2014;37(8):597-608. [CrossRef]
42. Kosmalski M, Deska K, Bąk B, Różycka-Kosmalska M, Pietras T. Pharmacological support for the treatment of obesity—present and future. *Healthcare (Basel)*. 2023;11(3):433. [CrossRef]
43. Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. Xenical in the prevention of diabetes in obese subjects (XENDOS) study. A randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Clin Diabetol*. 2004;5(2):95-104.
44. McElroy SL, Hudson JI, Capece JA, et al. Topiramate for the treatment of binge eating disorder associated with obesity: a placebo-controlled study. *Biol Psychiatry*. 2007;61(9):1039-1048. [CrossRef]
45. Li Z, Maglione M, Tu W, et al. Meta-analysis: pharmacologic treatment of obesity. *Ann Intern Med*. 2005;142(7):532-546. [CrossRef]
46. Haddock CK, Poston WS, Dill PL, Foreyt JP, Ericsson M. Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized clinical trials. *Int J Obes Relat Metab Disord*. 2002;26(2):262-273. [CrossRef]
47. Singh AK, Singh R. Pharmacotherapy in obesity: a systematic review and meta-analysis of randomized controlled trials of anti-obesity drugs. *Expert Rev Clin Pharmacol*. 2020;13(1):53-64. [CrossRef]
48. Nuffer WA, Trujillo JM. Liraglutide: a new option for the treatment of obesity. *Pharmacotherapy*. 2015;35(10):926-934. [CrossRef]
49. Elkind-Hirsch KE, Chappell N, Shaler D, Storment J, Bellanger D. Liraglutide 3 mg on weight, body composition, and hormonal and metabolic parameters in women with obesity and polycystic ovary syndrome: a randomized placebo-controlled-phase 3 study. *Fertil Steril*. 2022;118(2):371-381. [CrossRef]
50. Gibbons C, Blundell J, Tetens Hoff S, Dahl K, Bauer R, Baekdal T. Effects of oral semaglutide on energy intake, food preference, appetite, control of eating and body weight in subjects with type 2 diabetes. *Diabetes Obes Metab*. 2021;23(2):581-588. [CrossRef]
51. Zhang L, Zhang M, Zhang Y, Tong N. Efficacy and safety of dulaglutide in patients with type 2 diabetes: a meta-analysis and systematic review. *Sci Rep*. 2016;6(1):18904. [CrossRef]
52. Da Porto A, Casarsa V, Colussi G, Catena C, Cavarape A, Sechi L. Dulaglutide reduces binge episodes in type 2 diabetic patients with binge eating disorder: a pilot study. *Diabetes Metab Syndr*. 2020;14(4):289-292. [CrossRef]
53. Apovian CM, Aronne LJ, Bessesen DH, et al. Pharmacological management of obesity: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2015;100(2):342-362. [CrossRef]

54. Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC. Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs. *Obes Rev*. 2013;14(5):383-392. [CrossRef]
55. Shukla AP, Kumar RB, Aronne LJ. Lorcaserin Hcl for the treatment of obesity. *Expert Opin Pharmacother*. 2015;16(16):2531-2538. [CrossRef]
56. Jastreboff AM, Aronne LJ, Ahmad NN, et al. Tirzepatide once weekly for the treatment of obesity. *N Engl J Med*. 2022;387(3):205-216. [CrossRef]
57. Ahmad NN, Robinson S, Kennedy-Martin T, Poon JL, Kan H. Clinical outcomes associated with anti-obesity medications in real-world practice: a systematic literature review. *Obes Rev*. 2021;22(11):e13326. [CrossRef]
58. Carbone EA, Caroleo M, Rania M, et al. An open-label trial on the efficacy and tolerability of naltrexone/bupropion SR for treating altered eating behaviours and weight loss in binge eating disorder. *Eat Weight Disord*. 2021;26(3):779-788. [CrossRef]
59. Theriot J, Sabir S, Azadfard M. Opioid Antagonists. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020. PMID: 30725764

ATATURK UNIVERSITY PUBLICATIONS

Review

DOI: 10.5152/Pharmata.2023.1318972

Gut Microbiota and Its Importance for Our Health

Berkay BİLGİN
Hayrunisa HANCI

Department of Pharmaceutical Microbiology, Atatürk University, Faculy of Pharmacy, Erzurum, Turkey

ABSTRACT

In 450 BC, Hipocrates stated, "All diseases begin in the gut. When the gut is sick, the back part of the body is also sick." This sentence is explained with the term "microbiota" in human body. Microbiota is the environment where microorganisms such as fungi, bacteria, and virus live together in our body. These microorganisms are found in certain parts of our body, upper respiratory tract, gastrointestinal tract, urinary system, eyes, and skin. Microbiota has been shown to change according to drug use, nutrition, age, and environment. As a result of these changes, learning, memory, functioning of the psychological or immune system, drug metabolism, and hormonal changes may occur. The aim of this article is to explain the importance of intestinal microbiota which is our second brain and the importance of our health.

Keywords: Intestinal flora, microbiota, microorganism

INTRODUCTION

Microorganisms that are normally found in plants, animals, and humans and are a part of their lives are called "normal micro flora," and the microorganism found in the environment is called "environmental microflora." Normal microflora and environmental microflora are in constant interaction with each other. This interaction is sometimes beneficial, often harmful.[1]

Human gut is a habitat for various microorganisms such as bacteria, fungi, parasites, and viruses. After years of joint development, the human body has become a mutually beneficial symbiotic relationship with gut bacteria.[2,3] The terms microbiome and microbiota are often used. Microbiome refers to the genes carried by microorganisms living commensally with humans.[4,5] Microbiota organisms, which are symbiotic with the human body, form a complex microecological flora. This symbiosis can affect both systems. For example, a change in the amount and distribution of the intestinal microorganism population may affect the intestinal barrier function, increase the secretion of toxic substances, and reduce the secretion of beneficial substances in the human body, leading to enteral and other diseases.[2] Age and diet, in particular, can have a significant impact on gut microbiology. Numerous human and animal studies have shown that different diets can cause significant changes in the microbiota. Infection and disease can also have an adverse effect on the normal intestinal flora of the host, thereby causing detrimental effects on the host.[6-8] Microbiota also plays an important role in human disease and health. In humans, the microbiota, especially the gastrointestinal (GI) microbiota, acts by providing the necessary signals for disease and health conditions, the maturation of immune cells, and the normal development of immune system functions, by absorbing and fermenting undigested carbohydrates.[9] There are different ecosystems in our world, each of which creates its own species and communities. Likewise, there are different ecosystemic structures in a human body—skin, mouth, intestines, and reproductive organs—and every organ that has a connection with the outside world has its own characteristic microbe communities.[10] The microbiota has been colonized in many systems in the human body.[11] The interactions of microorganisms (microbiota) inside the oral cavity with their hosts is oral microbiota.[12] Skin microbiota refers to microorganisms typically found on human skin. Most of them consist of about 1000 different types of bacteria.[13,14] This flora prevents the colonization of pathogenic microorganisms on the skin surface by consuming the nutrients of transient pathogenic organisms, secreting chemicals against pathogenic microorganisms, or stimulating the immune system.[15] The vaginal microbiota is composed of especially lactobacilli and plays a very important role in maintaining the health of the female genital tract and preventing genitourinary infections.[16] However, since the digestive system has a very large surface area and contains rich nutrients for microorganisms, it offers the most suitable environment for colonization. Therefore, the colon alone contains more than 70% of the microorganisms in our body.[11]

Received: 23.06.2023
Accepted: 03.07.2023
Publication Date: 31.07.2023

Corresponding Author:
Hayrunisa HANCI
E-mail: hayrunisa.hanci@atauni.edu.tr

Cite this article as: Bilgin B, Hancı H. Gut microbiota and its importance for our health. *Pharmata* 2023;3(3):71-73.

The Relationship Between Flora and the Human Body

The fetus is sterile while in the uterus. The baby comes into contact with microorganisms during and immediately after birth. These microbes that settle on the skin and mucous membranes form the groups of microorganisms we call flora.[17] Normal microbial flora can be examined in 2 groups as permanent flora and temporary flora. Permanent flora is a community of microorganisms that are always present in certain body parts at a certain age, generally unchanged, that regenerate even if they are eliminated for a short time and that shows continuity. Temporary flora, in addition to the permanent flora, is a community of environmental microorganisms, most of which do not cause disease, can sometimes be pathogenic, and can remain from a few hours to several weeks.[18] The number of people living on earth is approximately 7 billion (as of 2012). On the other hand, the number of bacteria living on earth is 5 nonillion (5×10^{30}). There is approximately 1 quintillion (10^{18}) bacteria per every living person. There are approximately 100 trillion cells in a human body.[19] There are microorganisms that live in the flora 10 times more than that, and the majority of them are bacteria.[20]

Formation of Intestinal Flora

The adult human intestine is an organ rich in microorganisms, the number of which reaches about 100 trillion. The number of species in this microorganism pool is between 300 and 1000 and the microorganism weight is about 2 kg. The majority of the microbiota consists of eukaryotic fungi, viruses, and a few archaea, with colonies formed by bacteria along the intestinal line.[21] Our brain is affected by our intestines and even our GI tract.[22] This situation complies with psychiatrists' principle of opening or closing our appetite as our mood changes. Biologists describe this line of communication as the "brain–gut axis line."[23] Gut–brain axis is a 2-way communication system between the central nervous system (CNS) and the enteric nervous system (ENS) and intestinal communities.[24,25] An organism's microbiota can control its social attitude and ability to cope with stress, including the host's behavior. In our intestines, mainly firmicutes, bacteroidetes, *Actinobacteria*, *Proteobacteria*, *Fusobacteria,* and *Verrucomicrobio* bacteria families live.[23] However, the human gut microbiota contains more than 1000 species and more than 7000 subspecies.[26]

Intestine–Brain Axis and Its Relationship with Microbiota

The mechanisms behind the developing gut–brain axis are still unclear, but there are many hypotheses explaining the role of the immune system, bacterial metabolites, and endocrine effects in this axis.[27] In the gut–brain axis, molecular communication between the CNS and the GI tract is critical for maintaining healthy brain function, especially in the aging state.[28,29] Today, this axis is accepted as a bidirectional system that regulates the functions of 2complex organs under physiological conditions or becomes disordered under pathological conditions. Bi-directional communication between gut and brain is regulated by neuronal, endocrine, and immunological levels. The second brain, known as the "Enteric Nervous System," consists of neuronal sheaths embedded in the walls of the long tube of our intestines, or digestive tract, which extends about 9 m from the alimentary canal to the anus.[30] The ENS is sometimes called the "second brain" and actually arises from the same tissue as the CNS during embryonal development. The microbiotic composition of the gut is associated with maintenance of gut homeostasis, protection against pathogens, and an appropriate immune response.[31] Many environmental factors affect the gut microbiome. Geography, life cycle, mode of birth, infant feeding, stress, exercise, hygiene, infections, medications, and nutrition are some of these environmental factors. Worldwide, western diet and lifestyle changes have increased cardiovascular disease, cancer, and metabolic and allergic diseases. Nutrition has a major impact on the shape and structure of the intestinal and even farther uterine microbiome during the neonatal period and into adulthood.[32]

The GI tract system (GIS) is the largest immune organ. The GIS-related products may be neuropathogenic. The potential mechanisms of the effect of the microbiota on the CNS are summarized as follows: changes in microbial content, immune stimulation, neural pathways (via nerve vagus), tryptophan metabolism, serotonin, intestinal hormonal response, and bacterial metabolites. Short-chain fatty acids are formed as a result of fermentation in carbohydrate-heavy diets, and they affect brain functions by mixing with the systemic circulation. The nervus vagus connects the brain directly with the stomach and intestines like a broad highway. Hormonal, nervous, and, interestingly, bacterial changes in the intestines are transmitted to the brain via the vagus nerve.[31] Various cancers, asthma, autism, diabetes mellitus, insulin and metabolic syndrome, obesity, autoimmune diseases, multiple sclerosis, psychological problems, and many other diseases can occur as a result of intestinal–brain disorders.[33]

CONCLUSION

In conclusion, it is understood that microbiota is an indispensable element for our health. In particular, the gut microbiota manages our body like a second brain. Keeping the intestinal microbiota composition in the right balance is very important for a healthy life. There is a mutual and strong interaction between nutrition and gut microbiota. It is thought that a significant part of the differences in the intestinal microbial patterns of individuals can be explained by nutrition. It has been shown that nutrition can affect both intestinal microbiota composition and function by affecting microbial diversity, microbial taxonomy, genetic information, gene expressions, and enzyme activities. Further studies are needed in order to solve the mechanism of the effects of nutrition on the microbiota and to develop recommendations in this regard. The results obtained from current studies underline the importance of adequate and balanced nutrition in terms of energy and macronutrients. In addition, it is predicted that it will be beneficial to enrich the diet with plant-based foods containing fiber components, which are natural prebiotic sources, and fermented foods, which are natural probiotic sources.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.H., B.B.; Design - H.H., B.B.; Supervision - H.H., B.B.; Resources - H.H., B.B.; Writing Manuscript - H.H., B.B.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

REFERENCES

1. Kaplan NTC, *Milli Eğitim Bakanlığı Gıda Teknolojisi Gıda- Mikroorganizma İlişkisi*. Ankara; 2015:4.
2. Zhu X, Han Y, Du J, Liu R, Jin K, Yi W. Microbiota-gut-brain axis and the central nervous system. *Oncotarget*. 2017;8(32):53829-53838. [CrossRef]

3. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science*. 2005;308(5728):1635-1638. [CrossRef]
4. Yılmaz K, Altundiş M. Gastrointestinal microbiota and fecal transplantation. *Nobel Med*. 2017;13(1):9-15.
5. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. *Br J Nutr*. 2015;113(1)(suppl):S1-S5. [CrossRef]
6. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222-227. [CrossRef]
7. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-563. [CrossRef]
8. O'Mahony SM, Felice VD, Nally K, et al. Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience*. 2014;277:885-901. [CrossRef]
9. Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK, Host–bacterial symbiosis in health and disease. *Adv Immunol*. 2010;107:243-274. [CrossRef]
10. McFall-Ngai M, Hadfield MG, Bosch TCG, et al. Animals in a bacterial world a new imperative fort he life scienes. *Proc Natl Acad Sci USA*. 2013;110(9):3229-3236. [CrossRef]
11. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. *Proc Natl Acad Sci USA*. 1998;95(12):6578-6583. [CrossRef]
12. Arweiler NB, Netuschil L. The oral microbiota. In: Schwiertz A, ed. *Microbiota of the Human Body*. Switzerland: Springer; 2016:45-60. [CrossRef]
13. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324(5931):1190-1192. [CrossRef]
14. Pappas S. *Your Body Is a Wonderland of Bacteria. Science NOW*. American Association for the Advancement of Science; 2009. Available at: https://www.science.org/action/doSearch?AllField=your+body+is. Accessed June 22, 2023.
15. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? *Br J Dermatol*. 2008;158(3):442-455. [CrossRef]
16. Borges S, Silva J, Teixeira P. The role of lactobacilli and probiotics in maintaining vaginal health. *Arch Gynecol Obstet*. 2014;289(3):479-489. [CrossRef]
17. Joklik WK, Willett HP, Amos DB. Zinsser's microbiology. In: David T., et al., eds. Toledo, OH: Appleton-Century-Crofts, Inc; 1976:404.
18. Rosenwald AG, Arora GS, Madupu R, Roecklein-Canfield J, Russell JS. The human microbiome project: an opportunity to engage undergraduates in research. *Procedia Comput Sci*. 2012;9:540-549. [CrossRef]
19. McFall-Ngai M. Adaptive immunity: Care for the community. *Nature*. 2007;445(7124):153. [CrossRef]
20. Bianconi E, Piovesan A, Facchin F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol*. 2013;40(6):463-471. [CrossRef]
21. Biedermann L, Rogler G. The intestinal microbiota: its role in health and disease. *Eur J Pediatr*. 2015;174(2):151-167. [CrossRef]
22. Nardone DA. Collecting and analyzing data: doing and thinking. In: Walker HK, Hall WD, Hurst JW, eds. *The Man and the Opportunity Clinical Methods: the History, Physical, and Laboratory Examinations*. 3rd ed. Boston: Butterworths; 1990.
23. Khanna S, Tosh PK. A clinician's primer on the role of the microbiome in human health and disease. *Mayo Clin Proc*. 2014;89(1):107-114. [CrossRef]
24. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. *Neurogastroenterol Motil*. 2012;24(5):405-413. [CrossRef]
25. Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol*. 1977;31:107-133. [CrossRef]
26. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, Stability and resilience of the human gut microbiota. *Nature*. 2012;489(7415):220-230. [CrossRef]
27. Cryan JF, O'Mahony SMO. The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol Motil*. 2011;23(3):187-192. [CrossRef]
28. Mayer EA, Savidge T, Shulman RJ. Brain-gut microbiome interactions and functional bowel disorders. *Gastroenterology*. 2014;146(6):1500-1512. [CrossRef]
29. Rao M, Gershon MD. The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol*. 2016;13(9):517-528. [CrossRef]
30. Charrier B, Pilon N. Toward a better understanding of enteric gliogenesis. *Neurogenesis (Austin)*. 2017;4(1):e1293958. [CrossRef]
31. Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. *J Neurosci*. 2014;34(46):15490-15496. [CrossRef]
32. De Palma G, Collins SM, Bercik P. The microbiota gut-brain axis in functional gastrointestinal disorders. *Gut Microbes*. 2014;5(3):419-429. [CrossRef]
33. Seganfredo FB, Blume CA, Moehlecke M, et al. Weight-loss interventions and gut microbiota changes in overweight and obese patients: a systematic review. *Obes Rev*. 2017;18(8):832-851. [CrossRef]