THE EURASIA PROCEEDINGS OF HEALTH, ENVIRONMENT AND LIFE SCIENCES

ISRES Publishing

VOLUME 17 ICGEHES CONFERENCE
ISSN: 2791-8033
ISBN: 978-625-6959-75-0
ICGEHES 2025: 5th International Conference on General Health Sciences (ICGeHeS)
May 01-04, 2025
Trabzon, Türkiye
Edited by: Mehmet Ozaslan (Chair), Gaziantep University, Türkiye

ICGEHES 2025

5th International Conference on General Health Sciences (ICGeHeS)

Proceedings Book

Editor

Mehmet Ozaslan
Gaziantep University, Türkiye

ISBN: 978-625-6959-75-0

Published by the ISRES Publishing

Address: Askan Mah. Akinbey Sok. No: 5-A/Konya/Türkiye

Web: www.isres.org

Contact: isrespublishing@gmail.com

Dates: May 01-04, 2025

Location: Trabzon, Türkiye

https://2025.icgehes.net

About Editor

Prof Dr. Mehmet Ozaslan
Department of Biology, Gaziantep University, Türkiye
Website: mehmetozaslan.com
E-mail: ozaslanmd@gantep.edu.tr

Language Editor

Lecturer Ceren Dogan
School of Foreign Languages, Necmettin Erbakan University, Türkiye
Email: cerendogan@erbakan.edu.tr

CONFERENCE PRESIDENTS

Prof. Dr. Mehmet Özaslan - ISRES Türkiye & Gaziantep University, Türkiye
Prof. Dr. Merita Rumano - University of Tirana, Albania

SCIENTIFIC BOARD

Ahmet Ozaslan - Gazi University, Türkiye
Alexander Oleynik - Si Dnipropetrovsk Medical Academy, Ukraine
Aruna M Jarju - King Graduate School-Monroe College, United States
Betül Apaydın Yıldırım - Atatürk University, Türkiye
Cahit Bagci - Sakarya University, Türkiye
David Wortley, International Society of Digital Medicine, United Kingdom
Djamil Krouf - University of Oran, Algeria
Elena Lukianova - Rudn-University, Russia
Eva Trnova - Masaryk University, Czech Republic
Gergely Pápay - Szent István University, Hungary
Gulfaraz Khan - United Arab Emirates University, U.A.E
Harun Ulger - Erciyes University, Turkey
Ildikó Járdi - Szent István University, Hungary
Iwona Bodys-Cupak, Jagiellonian University Medical College, Poland
Jaleela Alawi - University of Bahrain, Bahrain
Karim Hajhashemi - James Cook University, Australia
Károly Penksza - Szent István University, Hungary
Khandakar A. S. M. Saadat- Gaziantep University, Türkiye
Kristina Kilova - Medical University Plovdiv, Bulgaria
Marinela Asenova Kozhuharova - Shterev Medical Center Reproductive Health, Bulgaria
Mariusz Jaworski - Medical University of Warsaw, Poland
Merita Rumano - University of Tirana, Albania
Mousa Attom- American University of Sharjah, U.A.E
Muhammad Safdar - Cholistan University, Pakistan
Mustafa Saber Al-Attar-Selahaddin University, Iraq
Nigora Muminova - Center for Professional Development of Medical Personal, Uzbekistan
Oltinoy Yakubova - Andijan State Medicine Institute, Uzbekistan
Pınar Gül - Ataturk University, Türkiye
Rıdwan Lawal, Lagos university, Nigeria
Rifat Morina - University of Prizren Ukshin Hoti, Kosovo
Sai Yan - Minzu University of China, China
Teuta Ramadani Rasimi, University of Tetova, Republic of Macedonia
Vladimir Protsenko - Rudn-University, Russia
Volodymyr Sulyma - Si Dnipropetrovsk Medical Academy, Ukraine
Yulduz Rasul-Zade - Tashkent Pediatric Medical Institute, Uzbekistan
Wenxia (Joyl Wu, Eastern Virginia Medical School, U.S.A.
Zhaklina Trajkovska-Anchevska - PHI University Clinic for Hematology-Republic of Macedonia

ORGANIZING COMMITTEE

Aynur Aliyeva - Institute of Dendrology of Anas, Azerbaijan
Dominika Falvai - Szent Istvan University., Hungary
Elman Iskender - Central Botanical Garden of Anas, Azerbaijan
Ishtar Imad - Rasheed University College, Iraq

Editorial Policies

ISRES Publishing follows the steps below in the procedings book publishing process.
In the first stage, the papers sent to the conferences organized by ISRES are subject to editorial oversight. In the second stage, the papers that pass the first step are reviewed by at least two international field experts in the conference committee in terms of suitability for the content and subject area. In the third stage, it is reviewed by at least one member of the organizing committee for the suitability of references. In the fourth step, the language editor reviews the language for clarity.

Review Process

Abstracts and full-text reports uploaded to the conference system undergo a review procedure. Authors will be notified of the application results in three weeks. Submitted abstracts will be evaluated on the basis of abstracts/proposals. The conference system allows you to submit the full text if your abstract is accepted. Please upload the abstract of your article to the conference system and wait for the results of the evaluation. If your abstract is accepted, you can upload your full text. Your full text will then be sent to at least two reviewers for review. **The conference has a double-blind peer-review process.** Any paper submitted for the conference is reviewed by at least two international reviewers with expertise in the relevant subject area. Based on the reviewers' comments, papers are accepted, rejected or accepted with revision. If the comments are not addressed well in the improved paper, then the paper is sent back to the authors to make further revisions. The accepted papers are formatted by the conference for publication in the proceedings.

Aims & Scope

Compared to other fields, developments and innovations in the fields of medical and health sciences are very fast. In this century, where the human population is rapidly increasing and technology is developing rapidly, health problems are constantly changing and new solutions are constantly being brought to these problems. With the Covid 19 epidemic, it has emerged that a health problem affects all humanity and all areas of life. For this reason, this conference focused on the changes and innovations in the field of Medical and Health Sciences.

The aim of the conference is to bring together researchers and administrators from different countries, and to discuss theoretical and practical issues of Medical and Health Sciences. At the same time, it is aimed to enable the conference participants to share the changes and developments in the field of Medical and Health Sciences with their colleagues.

Articles: 1- 9

CONTENTS

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 1-9

ICGeHeS 2025: International Conference on General Health Sciences

Non-Hereditary and Hereditary Risk Factors of Breast Cancer

Faten Alnoaimi
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Breast cancer is a worldwide health concern with the incidence rate increasing from year by year. Breast cancer is the most commonly diagnosed cancer among women and is the leading cause of cancer-related mortality among women in both developed and developing countries. Breast cancer initiation and progression is a complex, multistage process. Breast cancer is caused by inherited or acquired genetic changes in the somatic cells of the breast. In spite of significant advances in breast cancer research, there are still many unresolved concerns. Therefore, the investigation of risk factors associated with breast cancer continues to be a topic of interest and research. The main risk factors in the development of breast cancer may be unchangeable factors such as genetics, age and gender and may also be changeable factors such as those associated with lifestyle and environment. By recognizing breast cancer risk factors, it is possible to reduce the risk of developing this cancer and thus reduce the mortality associated with it, especially among women with these risk factors. This study aims to present the latest developments in the field of studying and identifying risk factors for breast cancer, as this topic is still one of the most important topics that are focused on reducing the risk of breast cancer and finding effective therapies to treat patients with this disease. To achieve this aim, we focused on the latest studies that have worked to identify risk factors for breast cancer. As a result, risk factors have been found to be either changeable or unchangeable. Changeable factors can be managed, while unchangeable factors are still being researched in order to find the most effective therapies to either prevent or control breast cancer.

Keywords: Breast cancer, Non-hereditary risk factors of breast cancer, Hereditary risk factors of breast cancer, Breast cancer genes

Introduction

Subdivide Cancer is a disease that occurs when a cell loses control of its normal growth and reproduction processes due to exposure to a factor that leads to a genetic mutation. Thereafter, the number of mutant cells increases and tumor forms as a result of cellular proliferation. Cancer cells have features that distinguish them from normal cells such as the ability to possess independent growth signals, lack of response to anti-growth signals, lack of response to apoptotic signals, and the ability to form new blood vessels, invade tissues, and metastasize (Açıkgoz & Akal -Yıldız, 2017). Breast cancer is caused by changes in the cells of the breast. Breast cancer often starts in the cells that line the milk ducts; therefore, this type is called ductal carcinoma. However, breast cancer may start in the lobules that house the milk-producing glands, therefore this type of cancer is called lobular carcinoma. Breast cancer receives the most attention compared to other cancers in women (Edward et al., 2021). According to World Health Organization (WHO) statistics in 2022, nearly 3 million new cases of breast cancer and 670,000 deaths were recorded globally. More than 99 % of these cases were among women (World Health Organization International Agency for Research on Cancer, 2024). In countries with a low human development index, 1 in 27 women are diagnosed and 1 in 48 women die due to breast cancer. This rate drops dramatically in countries with a high human development index, where one in 12 women will be diagnosed with breast cancer and one in 71 women will die (Momenimovahed & Salehiniya, 2019).

While there are many uncertainties surrounding the origin of breast cancer, there is evidence that the risk of developing breast cancer is related to many factors. Despite the variety of treatments available for breast cancer, the number of deaths associated with breast cancer remains high. For this reason, many specialized agencies are examining the risk factors associated with breast cancer in order to take action to reduce the incidence of breast cancer. Any substance, disease, or characteristic of an individual that increases the likelihood of developing breast cancer is a risk factor (Kashyap et al., 2022).

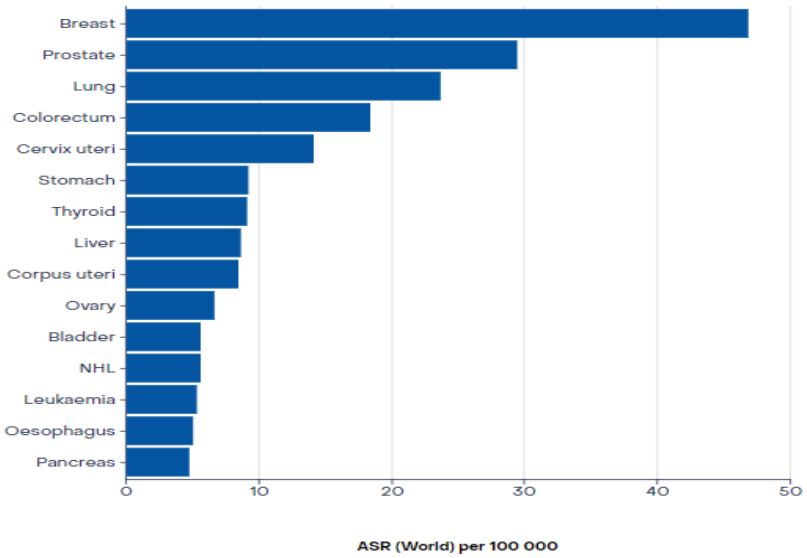

Figure 1. Breast cancer incidence rate compared to other types of cancer Source: (GLOBOCAN, 2022).

It has been shown that 5-10% of breast cancers are associated with non-modifiable risk factors such as genetic mutations and family history while 20-30% of breast cancers are associated with modifiable risk factors such as lifestyle and environmental factors. The presence of one or more of these risk factors will not necessarily lead to the development of breast cancer, thus some women carry one or more risk factors without developing the disease, while some women can develop breast cancer without carrying any of the risk factors. The reason for this is the genetic variability of individuals and the various forms of mutations they may have. In addition, the effect of the same substance varies in different people and the duration and severity of exposure plays an important role in the formation of breast cancer (Obeagu & Obeagu, 2024).

Risk Factors of Breast Cancer

Breast cancer risk factors are divided into non-hereditary and hereditary factors.

Non-Hereditary Risk Factors of Breast Cancer

Non-hereditary breast cancer risk factors are mostly related to environment and lifestyle.

Gender

The most important risk factor associated with breast cancer is being female, with 99% of breast cancer cases occurring in women. Male breast cancer is very rarely a rare disease (Yousef, 2017). Males may develop breast cancer due to many causes, including long-term exposure to radiation or a hormonal imbalance; however, the majority of male breast cancer cases are due to the BRCA2 gene mutation (Momenimovahed & Salehiniya, 2019). Male breast cancer differs from female breast cancer especially in terms of misdiagnosis and invasion. Men's lack of knowledge about breast cancer leads to late-stage diagnoses. Another reason why male breast cancer is worse than female breast cancer is that male breast tissue is thinner and tumor cells travel to the armpit and lymphatic vessels faster (Hassett et al., 2020).

Age

The incidence of breast cancer is directly proportional to age. The incidence of breast cancer peaks around age 50 due to menopause in women, but after that the rate may decrease or remain constant. However, young age is associated with a poor prognosis as the tumor appears large and at diagnosis, the lymph nodes are often positive and the recurrence rate is high (Momenimovahed & Salehiniya, 2019).

Age of Menarche and Menopause

It has been shown that women who have early menstrual periods are twice as likely to develop breast cancer compared to other women (Mao et al., 2023). On the other hand, menopause after the age of 55 also increases the risk of breast cancer (Kashyap et al., 2022). This is because women in this condition are exposed to sex hormones such as estrogen and progesterone for a long period of their lives. Late menarche or early menopause reduces the exposure of breast cells to female sex hormones and thus reduces the risk of breast cancer (Obeagu & Obeagu, 2024).

Pregnancy and Breastfeeding History

Increasing the number of births contributes to reducing the risk of breast cancer, as each birth reduces the risk of breast cancer by 10%. The reason for this is that complete lobular differentiation does not occur in women who have not given birth (Nicolis et al., 2024). The onset of breast cancer is inversely proportional to the degree of breast differentiation, with undifferentiated breasts leading to more aggressive tumors. In addition, the lack of differentiation of breast cells leads to increased susceptibility to non-estrogenic mutations and estrogen genotoxicity. Breastfeeding also increases the differentiation of breast cells, so breastfeeding for longer than 12 months reduces the risk of breast cancer (Anothaisintawee et al., 2013).

Contraceptive Methods, Ovulation-Stimulating Drugs and Postmenopausal Hormone Therapy

Despite the important role that female sex hormones and growth hormones play in the normal development of the breast and the formation of the mammary duct, exposure to high or prolonged levels of these hormones increases the risk of breast cancer (Łukasiewicz et al., 2021). Therefore, the use of birth control pills, ovulation-inducing drugs, and hormone replacement therapy for menopausal symptoms is associated with an increased risk of breast cancer (Westhoff & Pike, 2018).

Breast Diseases

Studies examining the relationship between breast disease and breast cancer are in progress. The breast can develop many diseases beyond breast cancer, such as breast cysts, fibroadenomas, and certain infections. Some breast disorders that cause breast lumps lead to health concerns associated with breast cancer. Some breast diseases may increase the risk of cancer by 4-5 times, but the association between benign breast disorders and breast cancer depends on the histologic classification of the disease and family history of breast cancer (Houghton & Hankinson, 2021).

Breast Density

The term breast density refers to the amount of dense tissue existing in the breast (Nazari & Mukherjee, 2018). Increased density of breast tissue is often associated with increased estrogen levels. Women with dense breast tissue have a higher risk of developing breast cancer than women with little or no dense breast tissue. The density of breast tissue is detected through a mammogram. Dense breasts may hide tumors because they appear white like tumors during mammography (Obeagu & Obeagu, 2024).

Smoking

Similar to all cancers, tobacco is a factor in the development of breast cancer in women who smoke. Studies have confirmed that women who smoke have a 10 times higher risk of developing breast cancer than women who non-smoke (Łukasiewicz et al., 2021). The carcinogenic effects of tobacco are caused by the presence of

aromatic hydrocarbons that lead to genetic polymorphisms in Nacetyltransferase-2 and consequently the development of breast cancer (Jones et al., 2017).

Consumption of Alcohol

Numerous studies have proven the link between alcohol consumption and an increased risk of breast cancer in women. Alcohol consumption accounts for 4% of breast cancer cases in developed countries. Studies show that ethanolic alcohol stimulates the growth of mammary epithelial cells and increases estradiol concentrations in the blood of premenopausal women.19 Ethanolic alcohol also enhances the carcinogenic effect of tobacco products and inhibits liver clearance (Łukasiewicz et al., 2021).

Obesity

Being overweight (BMI above 25 kg/m2) is a risk factor associated with breast cancer in women. Basically, estrogen is produced in the body by the ovaries, but after menopause, fat tissue produces a small amount of estrogen. More body mass means more fats tissue, which means higher estrogen levels and an increased risk of breast cancer (Tzenios, 2023). On the other hand, being overweight is associated with increased inflammation, which may increase the risk of breast cancer. In breast tissue containing excess fatty tissue, a higher dose of alcohol is metabolized into acetaldehyde, which binds to proteins and DNA and interferes with the antioxidant defense system, DNA synthesis and repair system by down-regulating BRCA1 (Vick et al., 2023).

Physical Activity

Physical inactivity is a risk factor associated with many types of cancer. Physical activity reduces the risk of chronic diseases as well as cancer and increases the chances of survival after being diagnosed with cancer (Goldschmidt et al., 2023). Physical activity supports breast cancer prevention, especially after menopause, by influencing steroid hormone metabolism, reducing inflammation, reducing body fat, improving immune system regulation, and altering the generation of free radicals. In addition, physical activity along with a balanced diet reduces the occurrence and development of genetic mutations associated with breast cancer, especially BRCA1/2 mutations (Fortner et al., 2023).

Other Factors

Several studies have demonstrated that many factors can increase the risk of developing breast cancer, such as type of diet, use of antiperspirants, stress, vitamin deficiencies, exposure to ionizers and other factors (Kashyap et al., 2022).

Hereditary Risk Factors of Breast Cancer

Many factors, including genetics, play a role in the initiation of breast cancer. Breast cancer is caused by genetic changes in the somatic cells of the breast. However, susceptibility to breast cancer may be hereditary. About 5-10% of breast tumors are hereditary, and another 15-20% are familial (Riedlova et al., 2020).

Family History and Genetic Predisposition

Personal history of breast cancer is a significant risk factor as there is an increased risk of recurrence in women who have experienced breast cancer previously. The second breast cancer may develop in the same or in a different breast. The occurrence of breast cancer among one or more close blood relatives indicates that breast cancer runs in the family (Obeagu & Obeagu, 2024). Studies have proved that the presence of breast cancer in a first-degree relative increases the risk of developing breast cancer by 2.1 times. In addition, the presence of breast cancer in the mother increases the risk by 2 times, in the sister by 2.3 times, and in the daughter by 1.8 times. If both the mother and sister have a history of breast cancer, the risk increases by 3.6 times (Açıkgöz & Akal Yıldız, 2017). Hereditary breast cancer is cancer that arises due to a genetic variation that is transmitted in an autosomal dominant manner. Breast cancer in males and breast cancer associated with cancer of the ovaries

or fallopian tubes in females is considered to be Hereditary Breast Cancer. Hereditary breast cancer was recognized as a syndrome in 1971 by Lynch and Crush. Genes associated with breast cancer risk are categorized by penetrance. Penetrance refers to the increased likelihood of breast cancer if a particular genotype is present (Mahdavi et al., 2019). In other words, penetrance means that the person carrying the mutation is susceptible to cancer (Wentzensen & Berg, 2018). Genes are divided into high, medium, and low penetrance genes according to their relative risk. High penetrance genes are associated with a relative risk of breast cancer higher than 5, and medium penetrance genes have a relative risk between 1.5 and 5. Low penetrance genes have a relative risk of less than 1.5. Accurate detection of breast cancer-associated genes can reduce the risk of cancer in susceptible people and their families (Riedlova et al., 2020).

High Penetrance Breast Cancer Genes

Highly penetrant genes are caused by mutations in a single cell or a small number of cells that lead to the formation of a pathological condition that may later turn into a malignant disease in the affected cell (Kang & Choi, 2021). Up to 25% of hereditary breast cancers are caused by a mutation in a rare but highly penetrant gene (BRCA1, BRCA2, PTEN, TP53, CDH1 and STK11). Mutations in one of these genes increase the lifetime risk of breast cancer by up to 80% (Shiovitz & Korde, 2015). The most common cause of hereditary breast cancers are BRCA1/2 genes whose mutations are inherited in an autosomal dominant manner (Naeem et al., 2019). Women who carry mutations in BRCA1 or BRCA2 are 10 to 20 times more likely to develop breast cancer than other women and are also more likely to be diagnosed with breast cancer at an earlier age. BRCA1/2 are two tumor suppressor genes by repairing double-stranded DNA breaks (Lilyquist et al., 2018). For this reason, cells carrying mutant BRCA genes are susceptible to DNA damage and genome instability and thus an increased risk of cancer (Feng et al., 2018). Males with breast cancer often carry mutations in the BRCA1 or BRCA2 gene. The most common mutations in males are BRCA2 compared to BRCA1 (Naeem et al., 2019). The TP53 gene is known as the guardian of the genome due to its important role in the process of gene regulation (McVeigh et al., 2021). The p53 protein is involved in cellular functions such as DNA repair, cell cycle control and apoptosis and therefore contributes to tumor suppression (Naeem et al., 2019). Mutations in TP53 lead to the development of multiple cancers (Li-Fraumeni syndrome) due to protein alterations, inactivation of DNA binding, or suppression of interaction with target genes (McVeigh et al., 2021). Females who carry a mutation in the P53T gene are most often diagnosed with breast cancer before the age of 30 (Bakhuizen et al., 2019). PTEN is a gene that contributes to tumor suppression by regulating PI3K and AKT signaling pathways. PTEN mutations are observed in cancer susceptibility syndromes and is the second most frequently mutated gene in human cancers after TP53. Women with Cowden syndrome who carry germline mutations in PTEN have a 50% lifetime risk of developing breast cancer (Ellsworth et al., 2019). STK11 is a gene that regulates energy metabolism and cell polarity, inhibits cellular proliferation, and interacts with the TOR pathway (Antov et al., 2017). People who carry a mutation in STK11 are susceptible to Peutz-Jeghers syndrome and various cancers, one of which is breast cancer where the lifetime risk of developing breast cancer is 24-54% (Lipsa et al., 2019). CDH1 gene is responsible for the CDH1 membrane protein that helps in the assembly of intercellular junctions and increases the adhesion of epithelial cells. Mutations in CDH1 gene lead to impaired attachment of cells to each other and to the basal membrane, which increases cell migration and invasion (Kim et al., 2016). Germline mutations in CDH1 have been associated with an increased lifetime risk of gastric and breast cancer (HDGC-syndrome) (Corso et al., 2020).

Moderate Penetrance Breast Cancer Genes

2%-3% of breast cancer cases are caused by a mutation in a moderately prevalent gene (Shiovitz & Korde, 2015). The most prominent genes with moderate penetrance are PALB2, ATM, CHEK2 and BRIP1. PALB2 is partner and localizer of BRCA2. PALB2 helps BRCA2 function because it is a key binding partner for BRCA2. In addition, PALB2 contributes to homologous recombination. PALB2 is highly associated with breast cancer and also causes aggressive clinicopathologic features (Wu et al., 2020). Breast cancer in males has been reported in male carriers of PALB2 variants (McVeigh et al., 2021). ATM is responsible for ataxia-telangiectasia syndrome and is also a gene associated with breast cancer. The ATM gene encodes a protein kinase that plays a key role in maintaining genomic stability as it acts in response to DNA damage, as well as in controlling the cell cycle and mitotic recombination (Marabelli et al., 2016). ATM gene is involved in cell cycle control, apoptosis, and gene regulation thus its dysregulation leads to many cancers such as breast cancer (Stucci et al., 2021). CHEK2 encodes a serine/threonine protein kinase. CHK2 is involved in DNA repair pathways, cell cycle regulation, and apoptosis. This gene also plays a role in the gene expression of the BRCA1/2 and TP53 genes. CHEK2 mutations are found in various types of cancer including breast cancer (Ansari et al., 2019). BRIP1

gene encodes the RecQ DEAH protein that interacts with BRCA1. In cooperation with the BRCA1 gene, the BRIP1 gene is involved in DNA damage repair and tumor suppression. Therefore, BRIP1 mutations lead to a failure to interact with BRCA1 resulting in a failure to repair damaged DNA. BRIP1 mutations have been associated with the development of tumors including breast cancer, ovarian cancer, and Fanconi anemia (Ouhti et al., 2016).

Low Penetrance Breast Cancer Genes

Gene mutations in high or medium penetrance genes are not exclusively responsible for genetic susceptibility to breast cancer; single nucleotide polymorphisms (SNPs) that may be found in the general population may also play a role in this susceptibility (Mahdavi et al., 2019). Low susceptibility alleles or modifier genes are polymorphic genes with specific alleles that are associated with altered risk of disease. In general, these variants are common in the population, and each variant can be said to be associated with only a small increase in an individual's breast cancer risk. However, the risk of these variants in the population as a whole is higher than rare, highly prevalent susceptibility genes (Ozgoz et al., 2020). Several susceptibility variants related to breast cancer were determined via genome-wide association studies (GWAS). GWAS is a dedicated research strategy to determine genetic correlations between genetic polymorphisms and diseases, traits, or variation (Mahdavi et al., 2019). Some variants in MSH2, MLH1, CYP1A1, NAT2, CAG, TGF-β1, LGR6, MDM4, TERT, ESR1, TOX, CASP8, MAP3K1, LSP1, FTO, FGFR2, RAD51C, RAD51D, MRPS30, COX 11 and SLC4A7 can all be considered low-penetrance genes for breast cancer. Further studies are still ongoing to identify more variants in other genes that are associated with breast cancer risk.

MicroRNAs (miRNAs)

Breast cancer susceptibility genes are not the only genetic factor that increases the risk of breast cancer. There are other genetic factors that play a role in increasing the risk of breast cancer such as miRNAs (Ellsworth et al., 2019). miRNAs are a group of non-protein-coding, endogenous, single-stranded and small RNAs that alter genetic expression through binding to messenger RNA (mRNA) domains and decreasing transcription or promoting mRNA degradation. Polymorphisms inside a germ line can lead to the removal or creation of mRNA binding domains or alter the specific functionality of the mRNA (Cardinali et al., 2022). Cancer-associated regions comprise approximately 50% of miRNAs -encoding genes. In addition, about one-third of protein-coding genes in humans are regulated by miRNAs. Thus, dysregulation of miRNAs can lead to the development in human cancers such as breast cancer. There are two types of miRNAs associated with breast cancer: Oncogenic miRNAs (oncomiRs) and tumor suppressor miRNAs (tsmiRs) (Loh et al., 2019). When oncomiRs overexpression occurs, breast cancer arises and the likelihood of cancer cell migration increases. This is because overexpression of RNA leads to suppression of the expression of tumor suppressor genes (e.g., miR-10b targeting E-cadherin, miR-21 targeting PDCD4, miR-155 and miR-27b targeting ST14). In contrast, tsmiRs inhibit the expression of some oncogenes, and therefore a decrease in their expression may lead to the development of breast cancer (e.g., miR-26b which reduces the expression of FOXM1 and miR-26b which targets CDK8) (Yang & Liu, 2020).

Mitochondrial DNA (mtDNA)

Mitochondria are involved in energy metabolism, cell proliferation, senescence, and apoptosis. Mitochondria contain their own circular DNA termed mitochondrial DNA (mtDNA). mtDNA contains 22 tRNA, 2 rRNA and 13 genes that encode the protein subunits of the mitochondrial complexes of the oxidative phosphorylation system (Weerts et al., 2019). The mtDNA content of each cell is constants but certain factors such as exposure to toxins, viral infections, and genetic mutations can lead to changes in it (Weerts et al., 2016). Genetic variants and oncogenic mutations in mtDNA may lead to the development of breast cancer (Weerts et al., 2019). In other words, low mtDNA gene expression or mtDNA mutations increase the risk of developing breast cancer as mtDNA mutations are found in 60% of breast tumors (Pérez-Amado et al., 2020).

Results and Discussion

Breast cancer is the most common cancer among women in both developed and developing countries. Several factors increase the risk of developing breast cancer. Identifying factors that increase the risk of breast cancer

contributes to increasing effective screening practices for individuals at risk and therefore reducing the incidence of breast cancer. Breast cancer risk factors are generally divided into two groups: hereditary and non-hereditary. Age, gender, lifestyle, harmful use of alcohol and tobacco, reproductive history, and use of hormone therapy are among the non-hereditary factors. Therefore, modifying lifestyle and daily habits may help reduce the incidence of breast cancer. However, women can develop breast cancer even though they do not carry any of the non-hereditary risk factors. This is because they may carry hereditary risk factors such as genetic mutations and a family history of the disease. Hereditary risk factors are unchangeable. Thus, women with a family history of breast cancer are advised to undergo genetic testing for counseling. Although not all women with breast cancer risk factors will develop breast cancer, knowing and studying risk factors is helpful in prevention.

Recommendations

Despite much research investigating the risk factors for breast cancer, many factors still need to be examined. Hereditary breast cancer risk factors are the most serious because they are often not amenable to lifestyle or environmental changes. Therefore, we recommend further research to identify hereditary breast cancer risk factors and the possibility of finding ways to help prevent breast cancer in individuals who are carriers of these risk factors.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* There is no fund for this article.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Acıkgoz, A., & Akal Yıldız, E. (2017). Meme kanseri etiyolojisi ve risk faktorleri etiology and risk factors of breast cancer. *Ergoterapi ve Rehabilitasyon Dergisi*, *5*(1), 45-56.

Anothaisintawee, T., Wiratkapun, C., Lerdsitthichai, P., Kasamesup, V., Wongwaisayawan, S., Srinakarin, J., ... & Thakkinstian, A. (2013). Risk factors of breast cancer: a systematic review and meta-analysis. *Asia Pacific Journal of Public Health*, *25*(5), 368-387.

Ansari, N., Shahrabi, S., Khosravi, A., Shirzad, R., & Rezaeean, H. (2019). Prognostic significance of CHEK2 mutation in progression of breast cancer. *Laboratory Medicine*, *50*(3), e36-e41.

Antov, G., Krasteva, M., Andonova, S., Savov, A., Angelova, S., Stoilov, L., & Toncheva, D. (2017). STK11 gene mutations among patients with sporadic breast cancer. *Genetika*, *49*(2), 399-413.

Bakhuizen, J. J., Hogervorst, F. B., Velthuizen, M. E., Ruijs, M. W., van Engelen, K., Van Os, T. A., ... & Ausems, M. G. (2019). TP53 germline mutation testing in early-onset breast cancer: Findings from a nationwide cohort. *Familial Cancer*, *18*, 273-280.

Cardinali, B., Tasso, R., Piccioli, P., Ciferri, M. C., Quarto, R., & Del Mastro, L. (2022). Circulating miRNAs in breast cancer diagnosis and prognosis. *Cancers*, *14*(9), 1-20.

Corso, G., Montagna, G., Figueiredo, J., La Vecchia, C., Fumagalli Romario, U., Fernandes, M. S., ... & Bonanni, B. (2020). Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: a multidisciplinary clinical review. *Cancers*, *12*(6), 1-25.

Edward, U., Obeagu, E.I., Okorie, H.M., Vincent, C. C. N., & Bot, Y.S. (2021). Studies of serum calcium, inorganic phosphate and magnesium levels in lactating mothers in Owerri. *Journal of Pharmaceutical Research International*, *33*(41B), 209-216.

Ellsworth, D. L., Turner, C. E., & Ellsworth, R. E. (2019). A review of the hereditary component of triple negative breast cancer: High-and moderate-penetrance breast cancer genes, low-penetrance loci, and the role of nontraditional genetic elements. *Journal of Oncology*, *2019*(1), 1-10.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., ... & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & Diseases*, *5*(2), 77-106.

Fortner, R. T., Brantley, K. D., Tworoger, S. S., Tamimi, R. M., Rosner, B., Farvid, M. S., ... & Eliassen, A. H. (2023). Physical activity and breast cancer survival: Results from the Nurses' Health Studies. *JNCI Cancer Spectrum*, *7*(1), 1-8.

Goldschmidt, S., Schmidt, M. E., & Steindorf, K. (2023). Long-term effects of exercise interventions on physical activity in breast cancer patients: A systematic review and meta-analysis of randomized controlled trials. *Supportive Care in Cancer*, *31*(2), 1-22.

Hassett, M. J., Somerfield, M. R., Baker, E. R., Cardoso, F., Kansal, K. J., Kwait, D. C., ... & Giordano, S. H. (2020). Management of male breast cancer: ASCO guideline. *Journal of Clinical Oncology, 38*(16), 1849-1863.

Houghton, S. C., & Hankinson, S. E. (2021). Cancer progress and priorities: breast cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *30*(5), 822-844.

Jones, M. E., Schoemaker, M. J., Wright, L. B., Ashworth, A., & Swerdlow, A. J. (2017). Smoking and risk of breast cancer in the generations study cohort. *Breast Cancer Research*, *19*, 1-14.

Kang, D., & Choi, J. Y. (2021). Breast cancer-related low penetrance genes. In *Translational research in breast cancer* (pp. 419-434). Singapore: Springer.

Kashyap, D., Pal, D., Sharma, R., Garg, V.K., Goel, N., Koundal, D., Zaguia, A., Koundal, S., & Belay, A. (2022). Global increase in breast cancer incidence: risk factors and preventive measures. *BioMed Research International*, *1*, 1–16.

Kim, S. A., Inamura, K., Yamauchi, M., Nishihara, R., Mima, K., Sukawa, Y., ... & Qian, Z. R. (2016). Loss of CDH1 (E-cadherin) expression is associated with infiltrative tumor growth and lymph node metastasis. *British Journal of Cancer*, *114*(2), 199-206.

Lilyquist, J., Ruddy, K. J., Vachon, C. M., & Couch, F. J. (2018). Common genetic variation and breast cancer risk—past, present, and future. *Cancer Epidemiology, Biomarkers & Prevention*, *27*(4), 380-394.

Lipsa, A., Kowtal, P., & Sarin, R. (2019). Novel germline STK11 variants and breast cancer phenotype identified in an Indian cohort of Peutz–Jeghers syndrome. *Human Molecular Genetics*, *28*(11), 1885-1893.

Loh, H. Y., Norman, B. P., Lai, K. S., Rahman, N. M. A. N. A., Alitheen, N. B. M., & Osman, M. A. (2019). The regulatory role of microRNAs in breast cancer. *International Journal of Molecular Sciences*, *20*(19), 4940.

Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. *Cancers*, *13*(17), 1-30.

Mahdavi, M., Nassiri, M., Kooshyar, M. M., Vakili-Azghandi, M., Avan, A., Sandry, R., ... & Gopalan, V. (2019). Hereditary breast cancer; Genetic penetrance and current status with BRCA. *Journal of Cellular Physiology*, *234*(5), 5741-5750.

Mao, X., Omeogu, C., Karanth, S., Joshi, A., Meernik, C., Wilson, L., Clark, A., Deveaux, A., He, C., Johnson, T., Barton, K., Kaplan, S., & Akinyemiju, T. (2023). Association of reproductive risk factors and breast cancer molecular subtypes: A systematic review and meta-analysis. *BMC Cancer*, *23*(1), 1-29.

Marabelli, M., Cheng, S. C., & Parmigiani, G. (2016). Penetrance of ATM gene mutations in breast cancer: A meta-analysis of different measures of risk. *Genetic Epidemiology*, *40*(5), 425-431.

McVeigh, U. M., Tepper, J. W., & McVeigh, T. P. (2021). A review of breast cancer risk factors in adolescents and young adults. *Cancers*, *13*(21), 1-30.

Momenimovahed, Z., & Salehiniya, H. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer: *Targets and Therapy*, *11*, 151–164.

Naeem, M., Hayat, M., Qamar, S. A., Mehmood, T., Munir, A., Ahmad, G., & Hussain, A. (2019). Risk factors, genetic mutations and prevention of breast cancer. *International Journal of Biosciences*, *14*(4), 492-496.

Nazari, S.S., & Mukherjee, P. (2018). An overview of mammographic density and its association with breast cancer. *Breast Cancer*, *25*, 259–267.

Nicolis, O., De Los Angeles, D., & Taramasco, C. (2024). A contemporary review of breast cancer risk factors and the role of artificial intelligence. *Frontiers in Oncology*, *14*, 1-18.

Obeagu, E.I., & Obeagu, G.U. (2024). Breast cancer A review of risk factors and diagnosis. *Medicine*, *103*(3), 1-6.

Ouhtit, A., Gupta, I., & Shaikh, Z. (2016). BRIP1, a potential candidate gene in development of non-BRCA1/2 breast cancer. *Front Biosci (Elite Ed)*, *8*(2), 289-298.

Ozgoz, A., Mutlu- Icduygu, F., Yukselturk, A., Samlı, H., Hekimler- Ozturk, K., & Baskan, Z. (2020). Low-penetrance susceptibility variants and postmenopausal oestrogen receptor positive breast cancer. *Journal of Genetics*, *99*, 1-10.

Pérez-Amado, C. J., Tovar, H., Gómez-Romero, L., Beltrán-Anaya, F. O., Bautista-Piña, V., Dominguez-Reyes, C., ... & Jiménez-Morales, S. (2020). Mitochondrial DNA mutation analysis in breast cancer: shifting from germline heteroplasmy toward homoplasmy in tumors. *Frontiers in Oncology*, *10*, 572954.

Riedlova, P., Janoutova, J., & Hermanova, B. (2020). Frequency of mutations in BRCA genes and other candidate genes in high-risk probands or probands with breast or ovarian cancer in the Czech Republic. *Molecular Biology Reports*, *47*(4), 2763-2769.

Shiovitz, S., & Korde, L. A. (2015). Genetics of breast cancer: a topic in evolution. *Annals of Oncology, 26*(7), 1291-1299.

Stucci, L. S., Internò, V., Tucci, M., Perrone, M., Mannavola, F., & Palmirotta, R. (2021). The ATM gene in breast cancer: its relevance in clinical practice. *Genes (Basel)*, *12*(5), 727.

Tzenios, N. (2023). Obesity as a risk factor for cancer. *EPRA International Journal of Research and Development (IJRD)*, 8(2), 101-104.

Vick, L. V., Canter, R. J., Monjazeb, A. M., & Murphy, W. J. (2023). Multifaceted effects of obesity on cancer immunotherapies: bridging preclinical models and clinical data. *Seminars in Cancer Biology*. 95, 88–102.

Weerts, M. J., Sieuwerts, A. M., Smid, M., Look, M. P., Foekens, J. A., Sleijfer, S., & Martens, J. W. (2016). Mitochondrial DNA content in breast cancer: Impact on in vitro and in vivo phenotype and patient prognosis. *Oncotarget*, *7*(20), 29166-29176.

Weerts, M. J., Sleijfer, S., & Martens, J. W. (2019). The role of mitochondrial DNA in breast tumors. *Drug Discovery Today*, *24*(5), 1202-1208.

Wentzensen, N., & Berg, C. D. (2018). Population testing for high penetrance genes: are we there yet?. *JNCI: Journal of the National Cancer Institute, 110*(7), 687-689.

Westhoff, C. L., & Pike, M. C. (2018). Hormonal contraception and breast cancer. *Contraception*, 98(3), 171–173.

World Health Organization International Agency for Research on Cancer. (2024, October 1). *Tables mode cancer &group_populations & multiple_populations*. Retrieved from https://gco.iarc.who.int

Wu, S., Zhou, J., Zhang, K., Chen, H., Luo, M., Lu, Y., ... & Chen, Y. (2020). Molecular mechanisms of PALB2 function and its role in breast cancer management. *Frontiers in Oncology*, *10*, 1-13.

Yang, Z., & Liu, Z. (2020). The emerging role of microRNAs in breast cancer. *Journal of Oncology*, *2020*(1), 1-7.

Yousef, A.J.A. (2017). Male breast cancer: Epidemiology and risk factors. *Seminars in Oncology*, *44*(4), 267-272.

Author(s) Information

Faten Alnoaimi
Gaziantep University
Üniversite Bulvarı 27310 Şehitkamil – Gaziantep, Türkiye
faten.alnoaimil@email.com

Mehmet Ozaslan
Gaziantep University
Universite Bulvarı 27310 Şehitkamil - Gaziantep, Türkiye

To cite this article:

Alnoaimi, F., & Ozaslan, M. (2025). Non-hereditary and hereditary risk factors of breast cancer. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 1-9.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 10-21

ICGeHeS 2025: International Conference on General Health Sciences

Molecular Docking Study of α-, β-, and γ-Mangostin from Mangosteen (*Garcinia mangostana* L.) Targeting VEGFR-2 and NRP-1 for Anti-Angiogenic Therapeutics in Retinopathy Diabetic

Melina Ayu Widiastuti
Universitas Gadjah Mada

Ganjar Andhulangi
Universitas Gadjah Mada

Supanji Supanji
Universitas Gadjah Mada

Abstract: To enhance the effectiveness of treatment in diabetic retinopathy patients, the development of drugs that have a combined effect of inhibiting Vascular Endothelial Growth Factor-2 (VEGFR-2) and Neuropilin-1 (NRP-1) should be conducted. This work simulates the interaction of α-mangostin, β-mangostin, and γ-mangostin (*Garcinia mangostana* L.) as a receptor against VEGFR-2 and NRP-1 as a ligand through the molecular docking approach. Redocking between receptor and native ligand (VEGFR-2, PDB ID: 4ASD and NRP-1 PDB ID: 5C7G) was performed using MGLTools 1.5.7 and Autodock Vina, then continued with PyMol2 to assure RMSD value of 0.981 Å for VEGFR-2 and 1.994 Å for NRP-1 (< 2 Å). The docking results showed that α-mangostin had the lowest binding energy to VEGFR-2 (-8.9 kcal/mol) and NRP-1 (-6.9 kcal/mol), followed by γ-mangostin with binding energy of -8.6 kcal/mol to VEGFR-2 and -6.9 kcal/mol to NRP-1, and β-mangostin with binding energy of -8.3 kcal/mol to VEGFR-2 and -6.5 kcal/mol to NRP-1. In comparison, the positive control, Sunitinib, showed binding energy of -7.9 kcal/mol to VEGFR-2 and -6.2 kcal/mol to NRP-1. This indicates that these compounds have a more lowest energy binding to VEGFR-2 and NRP-1 than Sunitinib. In addition, the docking results visualized using Biovia Discovery Studio 2021 showed that these compounds have hydrogen bonds and several other bonds to the active sites of VEGFR-2 and NRP-1. Hence, the proposed compounds have the potential to be further synthesized and evaluated in vitro and in vivo as a pathological anti-angiogenesis drug in diabetic retinopathy.

Keywords: VEGFR-2, NRP-1, Anti-angiogenic, Retinopathy diabetic

Introduction

Diabetic retinopathy (DR) is a severe microvascular complication of diabetes that can result in permanent blindness. The global prevalence of diabetic retinopathy is anticipated to rise from 126.6 million in 2010 to 191.0 million in 2030, with 30% of the population at risk of blindness (Zheng et al., 2012). Chua et al., (2017) reported that Indonesia has a significantly higher prevalence of diabetic retinopathy and vision-threatening diabetic retinopathy than other countries in the Asia-Pacific region, with rates of 43.1% and 26.3%, respectively.

Non-proliferative diabetic retinopathy (NPDR) is an early stage of diabetic retinopathy that is characterized by microaneurysms, small hemorrhages, and lipid exudates (Saravanan et al, 2013). If left untreated, NPDR may develop into Proliferative Diabetic Retinopathy (PDR), which is characterized by angiogenesis as a result of elevated reactive oxygen species (ROS) in vascular endothelial cells (Kaur et al, 2012; Giacco & Brownlee,

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

2010; Reddy et al, 2014). Through inflammation and pericyte mortality, this results in irreversible cell injury (Giacco & Brownlee, 2010; Reddy et al., 2014; El-Osta et al., 2008). Localized protrusion of the capillary wall results from the loss of pericytes, which is associated with the formation of microaneurysms. Leukocyte adhesion and infiltration increase in response to inflammation, as do pro-inflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α), and IL-6. Endothelial cells, neuroglial cells, and the blood-retina barrier are all damaged as a consequence (Miyamoto et al., 1999; Beltramo & Porta, 2013).

Damage to vascular endothelial cells, neuroglial cells and the blood-retina barrier leads to capillary occlusion and retinal ischemia. Hypoxia will result from these conditions, which activates Hypoxia-Inducible Factor-1α (HIF-1α) and increases the expression of Vascular Endothelial Growth Factor-A (VEGF-A) (Huang et al, 2014). VEGF-A interacts with the VEGFR-2 receptor, which can be further amplified by neuropilin-1 (NRP-1) to induce cell proliferation and migration through the mitogen-activated protein kinases (MAPKs) and phosphoinositide 3-kinase (PI3K)-Akt pathways (Shintani, et al., 2006; Koch & Claesson-Welsh, 2012; Simons et al., 2016, 2016; Zachary, 2011). This leads to the development of new blood vessels that are more susceptible to damage and vascular leakage, which exacerbates diabetic retinopathy. Additionally, the interaction between VEGF-A and VEGFR-2 disrupts the adherens and junctions of vascular endothelial cells, resulting in vascular hyperpermeability and fluid extravasation (Koch & Claesson-Welsh, 2012; Simons et al., 2016). This implies that, in diabetic retinopathy, angiogenesis is significantly influenced by VEGF-A signaling through VEGFR-2 and NRP-1.

Anti-VEGF drugs, including bevacizumab, ranibizumab and aflibercept, are commonly used in patients with diabetic retinopathy to prevent macular edema and retinal angiogenesis. Although these drugs are effective, some patients with diabetic retinopathy develop resistance and poor efficacy due to polymorphisms in the VEGF gene (El-Shazly et al, 2013). In addition, patient access to treatment has been limited by the exorbitant cost of anti-VEGF drugs (Zhao & Singh, 2018). Therefore, the research for new targets and therapies to improve the treatment efficacy in patients with diabetic retinopathy is still ongoing, one of which is the development of drugs targeting VEGFR-2. Sunitinib is one of many drugs that target VEGFR-2. It is commonly used in the treatment of renal cell carcinoma (RCC) and has been shown to be effective in inhibiting angiogenesis (Hao & Sadek, 2016). Therefore, in this study, sunitinib was chosen as a reference for the development of anti-angiogenesis drugs.

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit that is extensively cultivated in Indonesia, with a particular emphasis on Sumatra, Java, Bali, and West Nusa Tenggara (Poerwanto et al., 2008). In the pericarp, there are numerous xanthones, particularly α-mangostin, β-mangostin, and γ-mangostin, which function as antioxidants, anti-inflammatory, antimicrobial, and anti-cancer compounds (Evalina et al., 2018; El-Kenawy et al., 2018). Previous research has demonstrated that xanthones can impede the proliferation and migration of T24 cancer cells (Szkaradek et al., 2019). Cell proliferation and migration can be influenced by the activity of the kinase domain on VEGFR-2, which regulates molecular pathways important for cell growth and movement and mediates intracellular signalling. However, the compounds with potential as anti-angiogenesis agents that selectively target VEGFR-2 have not been thoroughly investigated.

Molecular docking is an extensively used in silico study that is used to predict the anti-angiogenic potential of phytopharmaceuticals. This method is time-saving, cost-effective, accurate, and rapid. Furthermore, this process can also help researchers estimate the interaction between the proposed compounds (ligand) and its target (receptor) (Ananto et al., 2024). This expedites the process of developing and discovering new drugs to treat diabetic retinopathy. Thus, the objective of this research is to examine the potential interaction of α-, β-, and γ-mangostin against VEGFR-2 and NRP-1 as anti-angiogenic drug candidates in diabetic retinopathy.

Methods

Protein Preparation

The three-dimensional (3D) crystal structures of VEGFR-2 (PDB ID: 4ASD) and NRP-1 (PDB ID: 5C7G) were derived from the Protein Data Bank (PDB). The Gasteiger values were calculated and all hydrogen atoms were appended to the protein 3D crystal structures using Autodock Vina software and MGL Tools 1.5.7 (Pham et al., 2022).

Ligand Preparation

The 3D structures of α-mangostin, β-mangostin, γ-mangostin, and Sunitinib were obtained in the SDF format from Pubchem. Then, the Cactus Online SMILES Translator-NCI/CADD was employed to convert the SDF format to PDB (Nicklaus et al., 2024). The command "set number or torsions" and the command "choose torsions" were employed to torsion ligands, which were subsequently encoded in the pdbqt format.

Docking Protocol

The docking simulation was conducted on a personal computer (PC) that was run on the Windows 11 Home 64-bit operating system (10.0, Build 22631) with an 11th generation Intel® Core™ i5-1135G7 processor @2.40GHz (8 CPUs) ~2.4GHz and 8192MB of RAM. Biovia Discovery Studio 2021, PyMol2, and Autodock Vina & MGL Tools 1.5.7 were employed to visualise ligand-receptor interactions, calculate RMSD, and for docking simulation, respectively. Redocking was performed between the protein and its native ligand at the active site of the target protein to conduct docking validation. The VEGFR-2 protein was docked using a 40 Å cubic grid box with 1000 Å spacing, while the NRP-1 protein was docked using a 40 Å cubic grid box with 0.375 Å spacing. The RMSD value that was approved was less than 2 (RMSD < 2). Setting coordinates for the VEGFR-2 protein were -23.259, 0.096, and -10.064 (x, y, and z), while the NRP-1 protein's setting coordinates were 13.375, -0.413, and 10.285 (x, y, and z), with an exhaustiveness value of 16. Based on the size and position of the cubic grid box for each protein, the original ligand was substituted with α-mangostin, β-mangostin, γ-mangostin, and Sunitinib in a similar protocol. By selecting the pose with the lowest binding affinity, the interaction was visualised after 20 poses were acquired.

Results and Discussion

Binding Pocket of Receptors with Native Ligands

Figure 1 illustrates 3D (left) and 2D (right) receptor's interactions of the native ligands, sorafenib to VEGFR-2 and bicine molecule to NRP-1. Various interactions occur between the amino acids of the receptor proteins and the ligands. These interactions include hydrogen bonds, hydrophobic interactions, and other interactions, as presented in Tables 3 for sorafenib to VEGFR-2 and Table 4 for bicine molecule to NRP-1. The binding energy and the stability of the receptor-ligand complex are significantly influenced by these interactions. Sorafenib forms important hydrogen bonds with amino acid residues such as ASP1046, VAL899, and GLU885, followed by the hydrophobic interactions, including LEU1019, VAL898, ALA866, VAL848, VAL916, and LYS868, as well as other interactions (halogen, pi-sigma, pi-sulfur, pi-pi T-shaped, etc.) on VEGFR-2. Meanwhile, the bicine molecule exhibits crucial hydrogen bonds with TRP29, SER74, and THR77. Therefore, these amino acid residues are regarded as critical binding sites in the active site of the receptor protein.

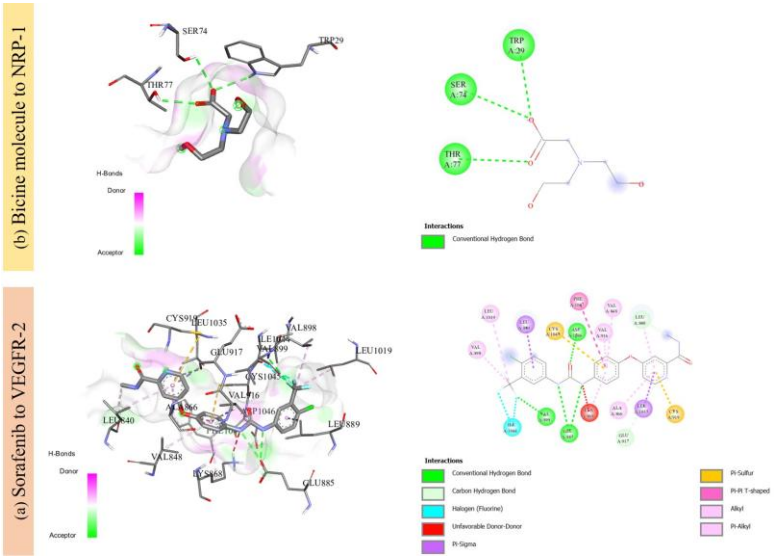

Figure 1. 3D (left) and 2D (right) visualization of native ligands: (a) Sorafenib to VEGFR-2 and (b) Bicine molecule to NRP-1.

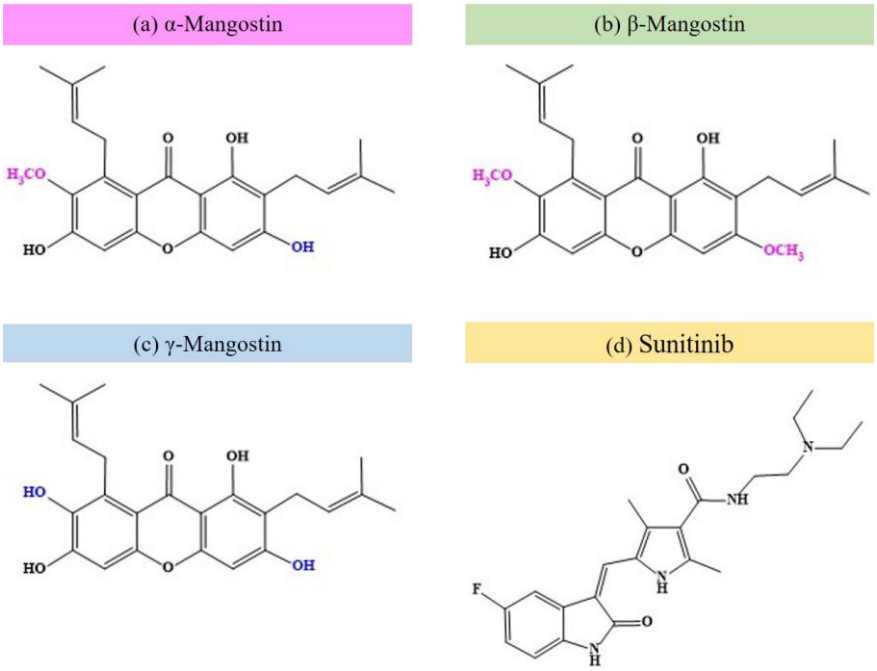

Figure 2. Proposed compounds of (a) α-mangostin, (b) β-mangostin, (c) γ-mangostin, and (d) Sunitinib as a positive control.

VEGFR-2 and NRP-1 are receptors involved in the signaling of angiogenesis in diabetic retinopathy. For this reason, there has been a recent surge in research that concentrates on their inhibition. The VEGFR-2 protein is comprised of three distinct structures: an extracellular domain, a transmembrane domain, and an intracellular domain, as reported by Go et al. (2023). Four regions form the intracellular domain: the juxtamembrane domain, kinase domain, kinase insert domain, and carboxyterminal domain (Manni et al., 2014). The juxtamembrane domain is responsible for the stabilization of the structure of VEGFR-2, whereas the kinase domain is responsible for the conformational changes of VEGFR-2 following activation (Manni et al., 2014). Both are involved in the process of signal transduction. Therefore, this protein targets the kinase domain and juxtamembrane.

NRP-1 is a transmembrane glycoprotein that functions as a co-receptor for VEGF-A and regulates the VEGFR-2 signaling pathway to improve the survival, proliferation, and chemotaxis of endothelial cells (Murga et al., 2004). NRP-1 is composed of three domains: the extracellular domain, transmembrane domain, and cytoplasmic domain (Li et al., 2011). The extracellular domain is composed of three regions: ala-2, b1-b2, and c domains (Uniewicz et al., 2014). Through interaction with the c-terminal arginine of VEGF, the b1 domain participates in the binding interaction with VEGF (Mota et al., 2018).

Structural Design of Ligands

Figure 2 shows the chemical structure of our proposed compounds, including α-, β-, and γ-mangostin, as well as sunitinib as a positive control compound. These proposed mangostin compounds as ligands consist of the xanthone nucleus as the main backbone with hydroxyl, methoxy, and isoprenyl groups as the main substituents, resulting in a variety of derivatives. The different properties exhibited by xanthones are highly dependent on the type and position of the substituents on the core ring, which is why they have been described as a 'privileged structure', as explained by Saraswathy et al. (2022). The xanthone structure and it's biological properties have generated a great deal of interest in these molecules. The hydroxyl group acts as a hydrogen acceptor, establishing hydrogen bonds with essential amino acids in VEGFR-2 and NRP-1. The xanthone backbone, methoxy and isoprenyl groups are designed to bind to the hydrophobic cavity and other interactions in the receptors.

We chose sunitinib, which is commonly used as a drug for cancer patients, as a positive control in this study. The structure of sunitinib consists of three parts, including a 5-fluoroindolin-2-one ring attached to a substituted pyrrole ring and connected to an attached amino side chain, as reported by AboulMagd and Abdelwahab (2021).

The amine and carbonyl groups of the indolin-2-one ring system can form a hydrogen bond with amino acids in VEGFR-2 and NRP-1. In addition, the indolin-2-one ring system, pyrrole ring connected to the amino side chain can lead to its incorporation into the hydrophobic and other interactions of the target protein. Therefore, this proposed compound was observed to evaluate the inhibitory activity against the target proteins VEGFR-2 and NRP-1.

Table 1. Docking validation between protein and native ligand.

Protein	PDB ID	Native ligand	Redocked RMSD (Å)
VEGFR-2	4ASD	Sorafenib	0.981
NRP-1	5C7G	Bicine molecule	1.994

In Silico Study: Binding Energy

In Table 1, the redocking protocol was employed to determine the RMSD values to be less than 2 Å as the docking validation, with the RMSD value in this study of 0.981 Å for the VEGFR-2 and 1.994 Å for the NRP-1. These values were generated using a specific grid box size. The RMSD is the most frequently employed method to assess the precision of the docking geometry by measuring the distance of the ligand from its reference point on the complex following the superposition of the receptor molecule (Kufareva & Abagyan, 2011). The grid box dimension of the docking method is deemed acceptable when the RMSD value is less than 2 (Wulan et al., 2023).

In Table 2, the binding energies of proposed compounds observed, with the ranges of -7.9 kcal/mol to -8.9 kcal/mol for VEGFR-2 and -6.2 kcal/mol to -6.9 kcal/mol for NRP-1. α-mangostin exhibited the lowest binding energies to VEGFR-2 and NRP-1 in comparison to sunitinib as positive control in this study. However, the binding energies of all proposed compounds did not apply equally to the VEGFR-2 when compared with the native ligand. Therefore, these proposed compounds formed a more stable complex with NRP-1 than bicine molecule. Overall, inhibitory activity of VEGFR-2 and NRP-1 is associated with compounds that have a lower binding energy and vice versa. This implies that their inhibitory activities were considerably greater than that of bicine molecule and that their interactions were more stable.

Protein-Ligand Interactions

In addition to binding energy, protein-ligand interactions important for VEGFR-2 and NRP-1 inhibition were used as screening criteria. Figure 3a-d shows the 3D (left) and 2D (right) representations of the different interactions between VEGFR-2 as receptor and the proposed compounds as ligands. In Table 3, the proposed compounds show hydrogen bond interactions between the amino acids ASP1046 and ASP814 in the VEGFR-2 protein to the O-H group on the xanthone skeleton of α-mangostin, ASP1046, LYS868 and ILE1025 to β-mangostin, ALA881 to γ-mangostin and CYS919 to the amine and carbonyl groups of the indolin-2-one ring system of sunitinib. In addition, LEU1049; ALA881; LEU882; VAL898; LEU889; VAL899; VAL916; CYS1045; ILE888; LEU1019; HIS1026; ILE1044; VAL899, ILE892; VAL898; ALA866; VAL848; LEU840; PHE1047; and LYS868 generally exhibited hydrophobic cavities with the xanthone skeleton, methoxy and isoprenyl groups of α-, β-, γ-mangostin, and formed interactions with the indolin-2-one ring system, pyrrole ring linked to the amino side chain of sunitinib. These proposed compounds also formed an additional electronic contact (pi-sigma, and carbon H-bond) with the α-, β-, γ-mangostin, with the sunitinib showed the pi-sigma, carbon H-bond, pi-sulfur, pi-pi T-shaped, and halogen.

Table 2. Binding energy of proposed compounds to VEGFR-2 and NRP-1

Compound	Binding energy (ΔG, kcal/mol)	
	VEGFR-2	NRP-1
α-mangostin	-8,9	-6,9
β-mangostin	-8,3	-6,5
γ-mangostin	-8,6	-6,9
Sunitinib	-7,9	-6,2
Sorafenib	-11,4	-
Bicine Molecule	-	-4,4

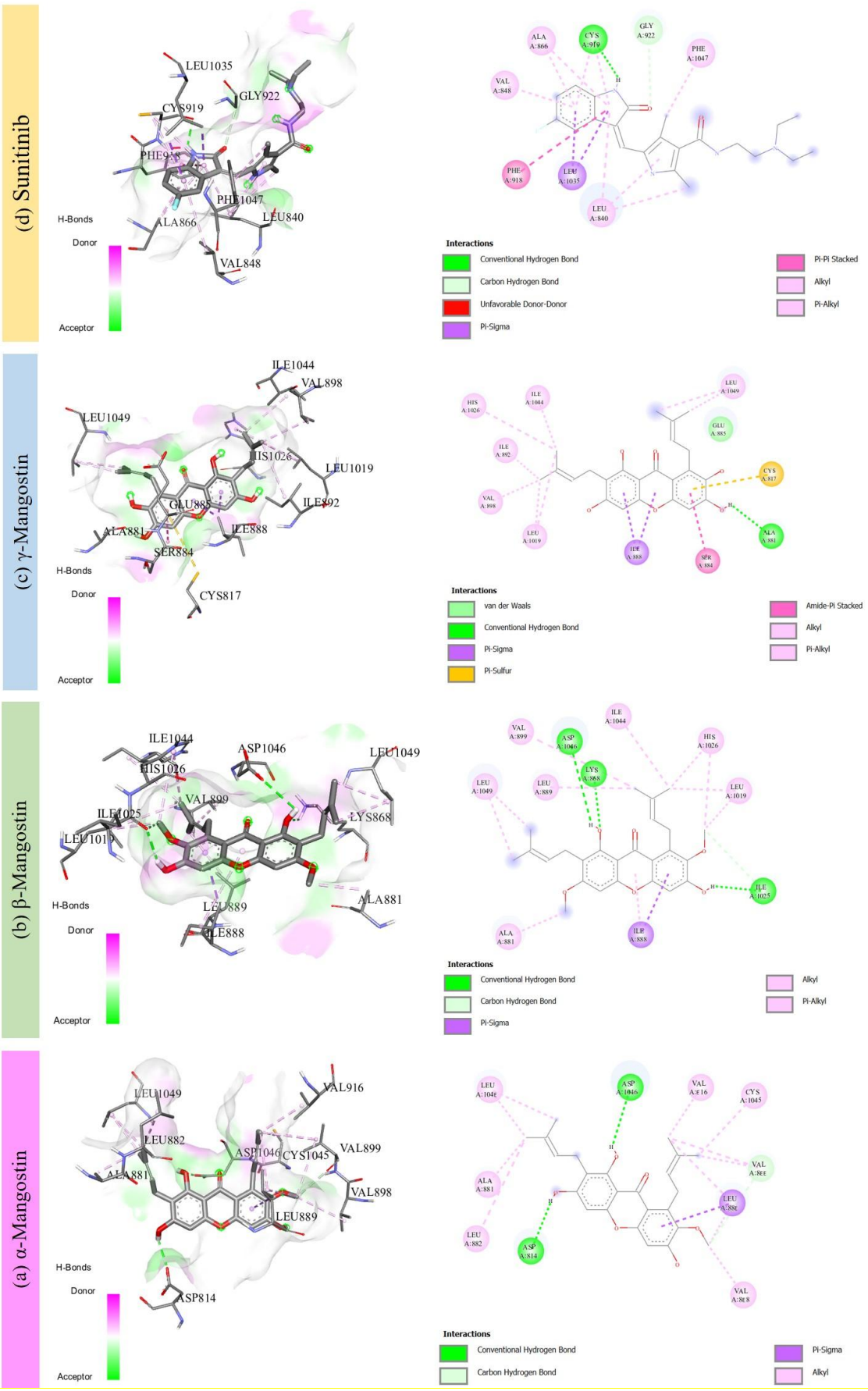

Figure 3. 3D (left) and 2D (right) visualization of (a) α-mangostin, (b) β-mangostin, (c) γ-mangostin, and (d) Sunitinib to VEGFR-2.

Table 3. Protein-ligan interaction of the proposed compound against VEGFR-2

Compound	Types of interaction		
	H-bond	Hydrophobic	Others
α-mangostin	ASP1046 (2.66 Å); ASP814 (2.29 Å)	LEU1049; ALA881; LEU882; VAL898; LEU889; VAL899; VAL916; CYS1045	Pi-sigma: LEU889 (3.71 Å) Carbon H-bond: VAL899 (3.40 Å)
β-mangostin	ASP1046 (2.88 Å) LYS868 (2.97 Å) ILE1025 (2.42 Å)	LEU1049; ALA881; ILE888; LEU1019; HIS1026; ILE1044; VAL899; LEU889	Pi-sigma: ILE888 (3.77 Å) Carbon H-bond: ILE1025 (3.33 Å)
γ-mangostin	ALA881 (2.30 Å)	ILE1044; HIS1026; ILE892; VAL898; LEU1019; LEU1049	Pi-sigma: ILE888 (3.91 Å; 3.87 Å) Pi-sulfur: CYS817 (5.75 Å)
Sunitinib	CYS919 (2.43 Å)	ALA866; VAL848; LEU840; PHE1047	Pi-sigma: LEU1035 (3.84 Å; 3.87 Å) Carbon H-bond: GLY922 (3.22 Å)
Sorafenib	ASP1046 (1.95 Å) VAL899 (2.89 Å) GLU885 (2.92 Å; 3.35 Å)	LEU1019; VAL898; ALA866; VAL848; VAL916; LYS868	Pi-sigma: LEU889 (3.61 Å); LEU1035 (5.74 Å) Pi-sulfur: CYS1045 (5.13 Å); CYS919 (5.33 Å) Pi-Pi T-shaped: PHE1047 (4.87 Å) Halogen: ILE1044 (3.16 Å; 3.70 Å) Carbon H-bond: LEU840 (5.39 Å); GLU917 (3.40 Å)

In Table 4, the proposed compounds show interactions with amino acid residues on NRP-1. α-mangostin formed three hydrogen bonds with TYR25, THR44 and TRP29, as well as a hydrophobic interaction with TYR25. In addition, the Pi-Pi T-shaped interactions formed at amino acids TYR25 and TRP29 indicated an interaction between the aromatic ring of the ligand and aromatic residues on the receptor. β-mangostin interacts with TRP29 and ILE143 via hydrogen bonding. TYR25 and TYR81 are involved in the Pi-Pi stacked interaction, which shows the interaction between the two aromatic ring systems of the ligand and the tyrosine residue. This interaction helps to increase binding stability through hydrophobic effects. TYR25 exhibits a Pi-Pi T-shaped interaction, which is a type of aromatic interaction that can increase the affinity of the ligand to the receptor through electrostatic interactions.

Table 4. Protein-ligan interaction of the proposed compound against NRP-1

Compound	Types of interaction		
	H-bond	Hydrophobic	Others
α-mangostin	TYR25 (2.72 Å); THR44 (2.51 Å) TRP29 (2.50 Å)	TYR25	Pi-Pi stacked: TYR25 (4.26 Å; 4.40 Å; 5.71 Å); TYR81 (4.90 Å); TRP29 (6.43 Å)
β-mangostin	TRP29 (2.83 Å) ILE143 (3.07 Å)	TYR25	Pi-Pi stacked: TRP29 (6.44 Å); TYR25 (5.51 Å; 4.37 Å; 4.45 Å); TYR81 (4.55 Å) Carbon H-bond: ASP48 (5.16 Å)
γ-mangostin	TYR25 (2.75 Å) THR44 (2.47 Å) TRP29 (2.51 Å)	TYR25	Pi-Pi stacked: TYR25 (4.27 Å; 4.41 Å; 5.72 Å); TYR81 (4.88 Å); TRP29 (6.43 Å)
Sunitinib	TYR81 (2.60 Å) THR77 (2.85 Å)	LYS79	Pi-Pi stacked: TRP29 (5.89 Å); TYR25 (5.41 Å) Pi-sigma: TYR25 (3.88 Å) Carbon H-bond: TRP29 (2.68 Å); LYS79 (3.68 Å)
Bicine molecule	TRP29 (2.78 Å) SER74 (2.75 Å) THR77 (2.67 Å)	-	-

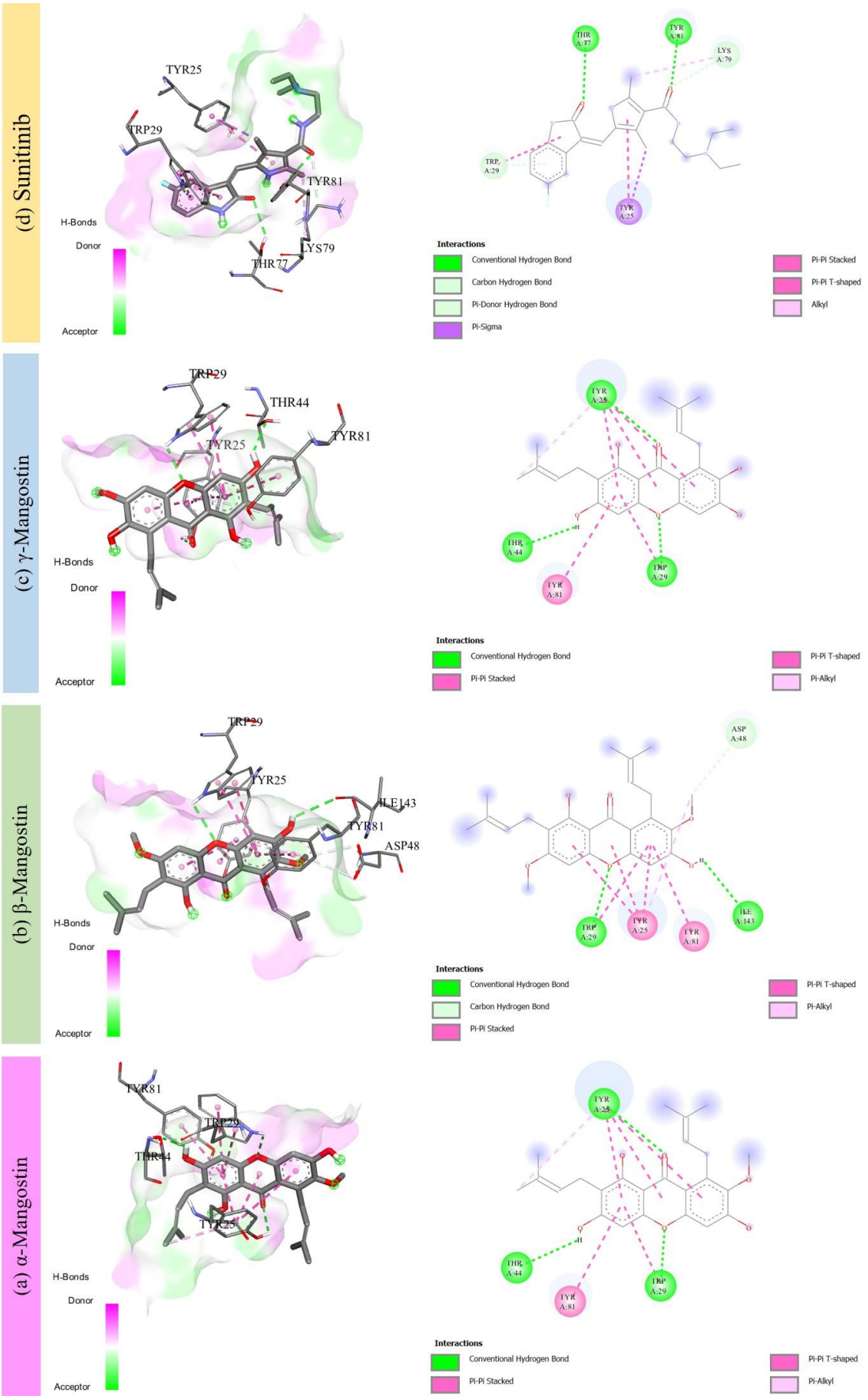

Figure 4. 3D (left) and 2D (right) visualization of (a) α-mangostin, (b) β-mangostin, (c) γ-mangostin, and (d) Sunitinib to NRP-1.

TYR25 also exhibits a Pi-Alkyl interaction, which involves the interaction of the aromatic ring of the ligand with the hydrocarbon side chain of a tyrosine residue. This interaction is hydrophobic in nature and helps to stabilise the ligand within the active site. γ-mangostin shows three hydrogen bonds with THR44, TYR25 and TRP29 and a hydrophobic interaction with TYR25. TYR81 and TYR25 form a pi-pi stacking interaction with the aromatic ring of the ligand. This interaction is important in stabilising the binding. TYR25 is also involved in Pi-Pi T-shaped interactions, which increase the stability of the complex through electrostatic interactions. Meanwhile, sunitinib forms two hydrogen bonds with TYR81 and THR77. Pi-Sigma and Pi-Pi stacked interactions with TYR25 played a role in binding stability, while alkyl interactions with LYS79 showed a hydrophobic contribution. Compared to sunitinib, α-mangostin showed the strongest interaction among other ligands.

This study shows that functional interactions in ligand-receptor complexes are strongly influenced by the presence of polar and hydrophobic amino acid residues interacting with ligands in the receptor active site. These interactions play an important role in stabilising the ligand-receptor complex and in determining the binding affinity of the ligand to the receptor. Therefore, compounds or ligands that are able to interact specifically with these key residues have the potential to inhibit the biological activity of the receptor protein and can therefore be developed as effective inhibitor candidates.

Conclusion

This study supports the hypothesis that α-, β- and γ-mangostin compounds derived from mangosteen fruit have potential as more potent inhibitors of VEGFR-2 and NRP-1 receptors compared to sunitinib. Molecular interaction analysis showed that the three compounds have a higher binding affinity to the active site of the receptor, and thus may be more effective in inhibiting VEGFR-2 and NRP-1 activity. With promising pharmacological properties, α-, β- and γ-mangostin can be developed as lead compounds in the design and optimization of VEGFR-2 and NRP-1 inhibitors for anti-angiogenesis therapy.

Recommendations

The docking results showed that α-, β- and γ-mangostins have high binding affinity towards VEGFR-2 and NRP-1. However, structural optimisation, such as side-chain modification, can be used to further increase the affinity and selectivity of the compounds towards the target receptors. Further validation is required to establish the pharmacological potential of these compounds, including molecular dynamics simulations to assess the stability of ligand-receptor complexes in a more realistic biological environment, experimental binding assays to determine the dissociation constant (Kd) of the ligand against VEGFR-2, and cell-based assays to assess the efficacy of angiogenesis inhibition. This study may be the first step in the development of α-, β- and γ-mangostin as anti-angiogenesis therapeutic candidates for patients with diabetic retinopathy.

Credit Authorship Contribution Statement

Melina Ayu Widiastuti: Writing – original manuscripts, Conceptualization, Methodology, Investigation, Data curation, Docking simulation, Funding acquisition, Project administration. **Supanji**: Writing – review & editing, Supervision, Formal analysis, Validation. **Ganjar Andhulangi**: Writing – review & editing, Docking Software, Visualization, Illustration, Formal analysis, Data curation, Resources, Validation.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Conflict of Interest

* No potential conflict of interest was declared by the authors.

Data Availability

* The authors affirm that the data that substantiates the results of this study is included in the paper. If raw data files are required, they can be obtained from the corresponding author upon a reasonable request.

Funding

* LPDP Indonesian Ministry of Finance Scholarship Fund for funding the participation to present the research at the 5th International Conference on General Health Sciences (ICGeHeS) 2025.

Acknowledgements

*This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May, 01-04, 2025.

References

AboulMagd, A. M., & Abdelwahab, N. S. (2021). Analysis of sunitinib malate, a multi-targeted tyrosine kinase inhibitor: A critical review. *Microchemical Journal*, *163,* 105926.

Ananto, A. D., Pranowo, H. D., Haryadi, W., & Prasetyo, N. (2024). Exploring the inhibition of SARS-COV-2 PLPRO: Docking and molecular dynamics simulation of flavonoid in red fruit papua and its derivatives. *Molekul*, *19*(3), 581.

Beltramo, E., & Porta, M. (2013). Pericyte loss in diabetic retinopathy: Mechanisms and consequences. *Current Medicinal Chemistry*, *20*(26), 3218–3225.

Chua, J., Lim, C. X. Y., Wong, T. Y., & Sabanayagam, C. (2017). Diabetic retinopathy in the Asia-Pacific. *Asia-Pacific Journal of Ophthalmology*, *7*(1), 3-16.

El-Kenawy, A. E., Hassan, S. M., & Osman, H. H. (2018). *Mangosteen* (*Garcinia mangostana* L (Vol.18,pp. 313–319). LWT.

El-Osta, A., Brasacchio, D., Yao, D., Pocai, A., Jones, P. L., Roeder, R. G., Cooper, M. E., & Brownlee, M. (2008). Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *The Journal of Experimental Medicine*, *205*(10), 2409–2417,

El-Shazly, S. F., El-Bradey, M. H., & Tameesh, M. K. (2013). Vascular endothelial growth factor gene polymorphism prevalence in patients with diabetic macular oedema and its correlation with anti-vascular endothelial growth factor treatment outcomes. *Clinical and Experimental Ophthalmology*, *42*(4), 369–378.

Evalina, G. M., Bambang, P., Bambang, S. S., Wahyu, W., & Arbi, D. (2018). Microstructural characterization of the *Garcinia mangostana* fruit at different maturity level. *Journal of Natural Remedies*, *18*(2), 63–70.

Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, *107*(9), 1058–1070.

Go, Y. J., Kalathingal, M., & Rhee, Y. M. (2023). Elucidating activation and deactivation dynamics of VEGFR-2 transmembrane domain with coarse-grained molecular dynamics simulations. *PLoS ONE*, *18*(2), e028178.

Hao, Z., & Sadek, I. (2016). Sunitinib: The antiangiogenic effects and beyond. *Oncotargets and Therapy*, *9*, 5495–5505.

Huang, H., He, J., Johnson, D., Wei, Y., Liu, Y., Wang, S., Lutty, G. A., Duh, E. J., Carmeliet, P., & Semba, R. D. (2014). Deletion of placental growth factor prevents diabetic retinopathy and is associated with AKT activation and HIF1α-VEGF pathway inhibition. *Diabetes*, *64*(1), 200–212.

Kaur, A., Kumar, R., & Sharma, A. (2024). Diabetic retinopathy leading to blindness- a review. *Current Diabetes Reviews*, *20*(9), e240124225997.

Kooi, C. W. V., Jusino, M. A., Perman, B., Neau, D. B., Bellamy, H. D., & Leahy, D. J. (2007). Structural basis for ligand and heparin binding to neuropilin b domains. *Proceedings of the National Academy of Sciences*, *104*(15), 6152–6157.

Kufareva, I., & Abagyan, R. (2011). Methods of protein structure comparison. *Methods in Molecular Biology, 857*, 231–257.

Li, J., Zhou, N., Luo, K., Zhang, W., Li, X., Wu, C., & Bao, J. (2014). In silico discovery of potential VEGFR-2 inhibitors from natural derivatives for anti-angiogenesis therapy. *International Journal of Molecular Sciences*, *15*(9), 15994–16011.

Li, X., Luo, F., Wang, S., Ni, E., Tang, X., Lv, H., Chen, X., Chen, L., & Yan, J. (2011). Monoclonal antibody against NRP-1 b1b2. *Hybridoma, 30*(4), 369–373.

Manni, S., Kisko, K., Schleier, T., Missimer, J., & Ballmer-Hofer, K. (2014). Functional and structural characterization of the kinase insert and the carboxy terminal domain in VEGF receptor 2 activation. *The FASEB Journal*, *28*(11), 4914–4923.

Miyamoto, K., Khosrof, S., Bursell, S., Rohan, R., Murata, T., Clermont, A. C., Aiello, L. P., Ogura, Y., & Adamis, A. P. (1999). Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proceedings of the National Academy of Sciences*, *96*(19), 10836–10841.

Mota, F., Fotinou, C., Rana, R. R., Chan, A. W. E., Yelland, T., Arooz, M. T., O'Leary, A. P., Hutton, J., Frankel, P., Zachary, I., Selwood, D., & Djordjevic, S. (2018). Architecture and hydration of the arginine-binding site of neuropilin-1. *FEBS Journal*, *285*(7), 1290–1304.

Murga, M., Fernandez-Capetillo, O., & Tosato, G. (2004). Neuropilin-1 regulates attachment in human endothelial cells independently of vascular endothelial growth factor receptor-2. *Blood*, 105(5), 1992–1999.

Nicklaus, M. C., Sitzmann, M., Filippov, I. V., Oellien, F., Bienfait, B., Voigt, J. H., & Sun, G. (2024, February 9). *Online SMILES translator*. Retrieved from https://cactus.nci.nih.gov/translate/.

Pham, Q. M., Le, T. T. H., Pham, T. H. M., Tran, Q. T., Lam, T., DO, Vu, T. T. L., & Pham, Q. L. (2022). Molecular docking tutorial using Autodock 4.2.6 on SARS-Cov-2 main protease for beginner. *Vietnam Journal of Science and Technology/Science and Technology*, *60(*6), 929–947.

Poerwanto, R., Efendi, D., Sobir, & Suhartanto, R. (2008) Improving productivity and quality of Indonesian mangosteen. *Acta Horticulturae*, *769*, 285-288.

Rahimi, N., & Costello, C. E. (2014). Emerging roles of post-translational modifications in signal transduction and angiogenesis. *Proteomics*, *15*(2–3), 300–309.

Rampogu, S., Baek, A., Park, C., Son, M., Parate, S., Parameswaran, S., Park, Y., Shaik, B., Kim, J. H., Park, S. J., & Lee, K. W. (2019). Discovery of small molecules that target vascular endothelial growth factor receptor-2 signalling pathway employing molecular modelling studies. *Cells*, 8(3), 269,

Reddy, M. A., Zhang, E., & Natarajan, R. (2014). Epigenetic mechanisms in diabetic complications and metabolic memory. *Diabetologia*, 58(3), 443–455.

Roth, L., Nasarre, C., Dirrig-Grosch, S., Aunis, D., Crémel, G., Hubert, P., & Bagnard, D. (2007). Transmembrane domain interactions control biological functions of neuropilin-1. *Molecular Biology of the Cell*, *19*(2), 646–654.

Saraswathy, S. U. P., Lalitha, L. C. P., Rahim, S., Gopinath, C., Haleema, S., SarojiniAmma, S., & Aboul-Enein, H. Y. (2022). A review on synthetic and pharmacological potential of compounds isolated from *Garcinia mangostana* linn. *Phytomedicine Plus*, 2(2), 100253.

Saravanan, V., Venkatalakshmi, B., & Rajendran, V. (2013). Automated red lesion detection in diabetic retinopathy. *IEEE Conference on Information & Communication Technologies (ICT)*, 6558096, 236–239,

Shintani, Y., Takashima, S., Asano, Y., Kato, H., Liao, Y., Yamazaki, S., Tsukamoto, O., Seguchi, O., Yamamoto, H., Fukushima, T., Sugahara, K., Kitakaze, M., & Hori, M. (2006). Glycosaminoglycan modification of neuropilin-1 modulates VEGFR2 signaling. *The EMBO Journal*, 25(13), 3045–3055.

Shintani, Y., Takashima, S., Kato, H., Komamura, K., & Kitakaze, M. (2009). Extracellular protein kinase CK2 is a novel associating protein of neuropilin-1. *Biochemical and Biophysical Research Communications*, *385*(4), 618–623.

Simons, M., Gordon, E., & Claesson-Welsh, L. (2016). Mechanisms and regulation of endothelial VEGF receptor signalling. *Nature Reviews Molecular Cell Biology*, *17*(10), 611–625.

Szkaradek, N., Sypniewski, D., Żelaszczyk, D., Gałka, S., Borzdziłowska, P., Marona, H., & Bednarek, I. (2019). Influence of new synthetic xanthones on the proliferation and migration potential of cancer cell lines in vitro. *Anti-Cancer Agents in Medicinal Chemistry*, *19*(16), 1949–1965.

Uniewicz, K. A., Ori, A., Ahmed, Y. A., Yates, E. A., & Fernig, D. G. (2014). characterisation of the interaction of Neuropilin-1 with heparin and a heparan sulfate mimetic library of heparin-derived Sugars. *PeerJ*, 2, e461.

Wulan, F. F., Wahyuningsih, T. D., Astuti, E., & Prasetyo, N. (2023). Towards targeting EGFR and COX-2 inhibitors: Comprehensive computational studies on the role of chlorine group in novel thienyl-pyrazoline derivative. *Journal of Biomolecular Structure and Dynamics*, *42*(19), 9857–9872,

Zachary, I. C. (2011). How neuropilin-1 regulates receptor tyrosine kinase signalling: the knowns and known unknowns. *Biochemical Society Transactions*, *39*(6), 1583–1591.

Zhao, Y., & Singh, R. P. (2018). The role of anti-vascular endothelial growth factor (Anti-VEGF) in the management of proliferative diabetic retinopathy. *Drugs in Context*, *7*, 1–10.

Zheng, Y., He, M., & Congdon, N. (2012). The worldwide epidemic of diabetic retinopathy. *Indian Journal of Ophthalmology*, *60*(5), 428,

Author(s) Information

Melina Ayu Widiastuti
Universitas Gadjah Mada
Biomedical Science, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada
Yogyakarta, 55281, Indonesia

Ganjar Andhulangi
Universitas Gadjah Mada
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada
Yogyakarta, 55281, Indonesia

Supanji Supanji
Universitas Gadjah Mada
Department of Ophthalmology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada
Yogyakarta, 55281, Indonesia
Contact e-mail: *supanji@ugm.ac.id*

To cite this article:

Widiastuti, M.A., Andhulangi, G., & Supanji, S. (2025). Molecular docking study of α-, β-, and γ-Mangostin from mangosteen (*Garcinia mangostana* L.) targeting VEGFR-2 and NRP-1 for anti-angiogenic therapeutics in retinopathy diabetic. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 10-21.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 22-26

ICGeHeS 2025: International Conference on General Health Sciences

Synthesis and Characterization of Silver Nanoparticles for Water Purification

Yasmeen Junejo
Cholistan University of Veterinary and Animal Sciences

Muhammad Safdar
Cholistan University of Veterinary and Animal Sciences

Mehmet Ozaslan
Gaziantep University

Abstract: Water contamination is a major global concern, necessitating the development of innovative and cost-effective purification technologies. Silver nanoparticles (AgNPs) have gained significant attention due to their remarkable antimicrobial properties, high surface area, and catalytic potential in water treatment. This study explores the synthesis, characterization, and application of AgNPs for water purification. Green synthesis using plant extracts, microbial processes, and chemical reduction methods ensures eco-friendly and sustainable nanoparticle production. The synthesized AgNPs are characterized using techniques such as UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and dynamic light scattering (DLS) to determine their size, morphology, and stability. The antimicrobial and pollutant-removal efficacy of AgNPs is evaluated against bacterial contaminants and heavy metals. Findings suggest that AgNPs exhibit strong bactericidal activity and high adsorption capacity, making them effective for water purification applications. However, concerns regarding toxicity, environmental impact, and large-scale production must be addressed. Future research should focus on optimizing synthesis techniques, enhancing stability, and ensuring safe disposal of AgNPs to minimize ecological risks. The integration of AgNP-based filtration systems with existing purification technologies offers a promising approach for sustainable and efficient water treatment solutions.

Keywords: Nanoparticle, Water Purification, Synthesis, Characterization

Introduction

Water scarcity and contamination are pressing global issues, with the World Health Organization estimating that by 2025, half the world's population will live in water-stressed regions (Zhang & Liu, 2025). Contaminants such as bacteria, viruses, heavy metals, and organic pollutants threaten water quality, necessitating advanced purification technologies (Chen & Wang, 2021). Silver nanoparticles (AgNPs) have garnered attention for their oligodynamic properties, enabling effective microbial disinfection and pollutant degradation (Li & Zhang, 2022). Their high surface-to-volume ratio enhances adsorption and catalytic activity, making them ideal for water treatment applications (Al-Haddad & Al-Sayed, 2022).

Some researchers have contributed significantly to the field, particularly in synthesizing and characterizing metal nanoparticles for environmental applications (Safdar et al., 2020; Junejo et al., 2019). Their work on AgNPs and platinum nanoparticles highlights the potential of green synthesis and antimicrobial applications. This article synthesizes their findings with recent advancements (2020–2025), focusing on AgNP synthesis, characterization, and water purification efficacy, while addressing challenges and future directions.

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

Synthesis of Silver Nanoparticles

Green Synthesis

Green synthesis leverages biological agents such as plant extracts and microorganisms to produce AgNPs, offering an eco-friendly alternative to chemical methods (Dutta & Sharma, 2024). Plant-based synthesis utilizes phytochemicals as reducing and capping agents, ensuring biocompatibility and reduced toxicity (Ahmad et al., 2020). For instance, *Terminalia chebula* fruit extract was used to synthesize AgNPs with a crystalline size of 21–24 nm, demonstrating significant antimicrobial and photocatalytic activity (Ghaffar & Farrukh, 2020).

Microbial synthesis, using bacteria like *Pseudomonas* sp., involves extracellular or intracellular reduction of silver ions, stabilizing nanoparticles with capping proteins (Zhao & Zhou, 2022). Junejo et al. (2019) explored tobramycin-stabilized AgNPs, achieving nanoparticles with enhanced antibacterial activity against pathogenic bacteria, highlighting the potential of biologically mediated synthesis.

Chemical Synthesis

Chemical reduction remains a widely used method, employing reducing agents like sodium borohydride or citrate to convert silver nitrate ($AgNO_3$) into AgNPs (Sharma & Yadav, 2023). This approach allows precise control over size and morphology but often involves toxic chemicals. Safdar et al. (2020) synthesized platinum nanoparticles using doxycycline as a reducing agent, a method adaptable to AgNPs, achieving sizes of 10–20 nm with high crystallinity. Chemical methods are scalable but require careful waste management to mitigate environmental impact (Singh & Mijakovic, 2024).

Factors Influencing Synthesis

Key parameters affecting AgNP synthesis include precursor concentration, pH, temperature, and reaction time. Higher pH and temperature typically reduce particle size, enhancing stability (Dutta & Sharma, 2024). The choice of reducing agent and stabilizer influences morphology, with spherical nanoparticles being most common for water purification due to their uniform surface area (Ahmad et al., 2020).

Characterization of Silver Nanoparticles

Characterization is critical to understanding AgNP properties and ensuring their efficacy in water purification (Zhang & Liu, 2025). Common techniques include:

- **UV-Vis Spectroscopy**: Confirms AgNP formation through surface plasmon resonance (SPR) peaks at 418–420 nm. Safdar et al. (2020) used UV-Vis to verify platinum nanoparticle formation at 264 nm, a technique applicable to AgNPs.
- **X-ray Diffraction (XRD)**: Assesses crystallinity and phase purity. AgNPs synthesized with *Terminalia chebula* exhibited crystalline sizes of 21–24 nm (Ghaffar & Farrukh, 2020).
- **Scanning Electron Microscopy (SEM)**: Reveals morphology and size distribution. Biologically synthesized AgNPs are often spherical, with sizes ranging from 5–50 nm (Ahmad et al., 2020).
- **Dynamic Light Scattering (DLS)**: Measures hydrodynamic size and stability, critical for assessing aggregation in aqueous environments (Zhao & Zhou, 2022).
- **Fourier Transform Infrared Spectroscopy (FT-IR)**: Identifies functional groups from capping agents, confirming stabilization (Dutta & Sharma, 2024).

These techniques ensure AgNPs meet the size (ideally <50 nm) and stability requirements for effective water treatment (Singh & Mijakovic, 2024).

Applications in Water Purification

Antimicrobial Activity

AgNPs exhibit strong bactericidal activity by disrupting bacterial cell membranes, releasing silver ions, and generating reactive oxygen species (ROS) (Li & Zhang, 2022). Studies show AgNPs achieve over 99.9% reduction in *E. coli* and other pathogens, making them effective for point-of-use water disinfection (Al-Haddad & Al-Sayed, 2022). Junejo et al. (2019) demonstrated that tobramycin-stabilized AgNPs inhibited Gram-positive and Gram-negative bacteria, with zones of inhibition comparable to standard antibiotics.

Pollutant Removal

AgNPs degrade organic pollutants and adsorb heavy metals due to their high surface area and catalytic properties (Baranwal & Kumar, 2021). Under visible light, AgNPs catalyze the degradation of dyes like methylene blue, achieving up to 92% removal (Ghaffar & Farrukh, 2020). They also remove heavy metals like mercury (Hg^{2+}) through adsorption and reduction, as shown in sensor applications (Kumar & Mathur, 2021).

Integration with Membranes

AgNP-impregnated membranes enhance filtration by combining antimicrobial and adsorptive properties (Bharti & Mukherji, 2023). Polysulfone membranes with AgNPs show improved biofouling resistance and virus removal, offering a scalable solution for water treatment (Wang & Chen, 2021).

Challenges and Limitations

Despite their promise, AgNPs face several challenges:

- **Toxicity**: High concentrations of AgNPs may harm aquatic ecosystems and human health through bioaccumulation (Li & Zhang, 2022).
- **Environmental Impact**: Improper disposal of AgNPs can contaminate water bodies, necessitating lifecycle assessments (Singh & Mijakovic, 2024).
- **Scalability**: Green synthesis methods are less scalable than chemical approaches, requiring optimization for industrial applications (Dutta & Sharma, 2024).
- **Stability**: AgNPs may aggregate in complex water matrices, reducing efficacy. Surface functionalization can mitigate this issue (Zhao & Zhou, 2022).

Future Directions

Future research should focus on:

- **Optimizing Green Synthesis**: Developing reproducible, scalable biological methods to reduce costs and environmental impact (Sharma & Yadav, 2023).
- **Enhancing Stability**: Using biocompatible stabilizers to prevent aggregation in diverse water conditions (Zhao & Zhou, 2022).
- **Toxicity Mitigation**: Designing AgNPs with controlled release of silver ions to minimize ecological risks (Li & Zhang, 2022).
- **Hybrid Systems**: Integrating AgNPs with carbon nanotubes or graphene for enhanced adsorption and disinfection (Baranwal & Kumar, 2021).

- **Regulatory Frameworks**: Establishing guidelines for safe AgNP use and disposal in water treatment (Zhang & Liu, 2025).

Conclusion

Silver nanoparticles offer a transformative approach to water purification, leveraging their antimicrobial and catalytic properties (Al-Haddad & Al-Sayed, 2022). Contributions from Safdar and Junejo (2010–2024) underscore the potential of green synthesis and precise characterization in developing effective AgNPs (Safdar et al., 2020; Junejo et al., 2019). While challenges like toxicity and scalability persist, ongoing advancements in synthesis, stabilization, and integration with filtration systems pave the way for sustainable water treatment solutions (Bharti & Mukherji, 2023). By addressing environmental concerns and optimizing production, AgNPs can play a pivotal role in ensuring clean water access globally (Zhang & Liu, 2025).

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* There is no fund for this article.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Ahmad, S., Munir, S., & Zeb, N. (2020). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract and their antibacterial activity. *Journal of Nanomaterials*, *2020,* 8910587.

Al-Haddad, A., & Al-Sayed, M. (2022). Silver nanoparticles for water disinfection: Mechanisms and applications. *Environmental Science and Pollution Research, 29*(4), 5123–5135.

Baranwal, A., & Kumar, A. (2021). Biogenic silver nanoparticles for enhanced photocatalytic degradation of organic dyes in water treatment. *Chemosphere*, *276*, 130156.

Bharti, S., & Mukherji, S. (2023). Silver nanoparticle-impregnated membranes for biofouling control in water purification. *Journal of Membrane Science*, *685,* 121948.

Dutta, V., & Sharma, S. (2024). Green synthesis of silver nanoparticles using plant extracts for sustainable water treatment. *International Journal of Biological Macromolecules*, *274,* 133211.

Ghaffar, N., & Farrukh, M. A. (2020). Green synthesis of silver nanoparticles using *Terminalia chebula* extract and their photocatalytic activity. *Materials Today: Proceedings, 29,* 102–108.

Junejo, Y., Safdar, M., & Akhtar, M. A. (2019). Synthesis and characterization of tobramycin-stabilized silver nanoparticles for antibacterial applications. *Journal of Inorganic and Organometallic Polymers and Materials*, *29*(1), 111–120.

Kumar, P., & Mathur, N. (2021). Silver nanoparticles for heavy metal removal from wastewater. *Environmental Nanotechnology, Monitoring & Management*, *16*, 100523.

Li, Y., & Zhang, X. (2022). Antimicrobial mechanisms of silver nanoparticles in water purification. *Nanomaterials*, *12*(21), 3707.

Safdar, M., Ozaslan, M., Khailany, R. A., & Junejo, Y. (2020). Synthesis, characterization, and applications of doxycycline-stabilized platinum nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials, 30*(6), 2430–2439.

Sharma, A., & Yadav, R. (2023). Advances in silver nanoparticle synthesis for environmental applications. *Bulletin of the National Research Centre*, *47*, 12.

Singh, P., & Mijakovic, I. (2024). Eco-friendly synthesis of silver nanoparticles for water treatment: Current trends and future perspectives. *Plasmonics*, *19*(2), 345–360.

Wang, L., & Chen, Y. (2021). Silver nanoparticles in water purification: Opportunities and challenges. *Journal of Environmental Chemical Engineering*, *9*(5), 105987.

Zhang, H., & Liu, Q. (2025). Nanomaterials for drinking water purification: A review of recent advances. *Nanomaterials*, *15*(1), 1707.

Zhao, X., & Zhou, Y. (2022). Sustainable synthesis of silver nanoparticles using microbial agents for water treatment. *ACS Sustainable Chemistry & Engineering*, *10*(12), 3890–3900.

Author(s) Information

Yasmeen Junejo
Cholistan University of Veterinary and Animal Sciences
Bahawalpur, Pakistan
Contact e-mail: *yasmeen@cuvas.edu.pk*

Muhammad Safdar
Cholistan University of Veterinary and Animal Sciences
Bahawalpur, Pakistan

Mehmet Ozaslan
Gaziantep University
Gaziantep, Türkiye

To cite this article:

Junejo, Y., Safdar, M., & Ozaslan, M. (2025). Synthesis and characterization of silver nanoparticles for water purification. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 22-26.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 27-34

ICGeHeS 2025: International Conference on General Health Sciences

Acute ^{60}Co Γ-Irradiation Effect on Lipid Peroxidation and Immune System in Experimental Animals

Oralbek Ilderbayev
L.N. Gumilyov Eurasian National University

Kerim Mutig
I.M. Sechenov First Moscow State Medical University

Gulzhan Ilderbayeva
L.N. Gumilyov Eurasian National University

Darkhan Uzbekov
L.N. Gumilyov Eurasian National University

Aruzhan Ilderbayeva
University of Duisburg-Essen

Ainur Zhanilova
L.N. Gumilyov Eurasian National University

Abstract: The objective of the study was to explore the biochemical and immunological indicators in experimentally irradiated animals. Goup I - intact, group II - exposed to γ-radiation with a dose 6 Gy. The parameters characterizing the body's immune system were studied, lipid peroxidation outputs and antioxidant protective enzymes activity in blood lymphocytes were identified. The results of the study showed that exposure to a sublethal dose of radiation entails a reduction in cellular immunity, especially T-lymphocytes and their subpopulation, as well as to a decrease in the functional activity of the body's non-specific defense. Leukopenia and lymphopenia were observed in irradiated animals. The same pattern was revealed for T-lymphocytes: the number decreased by 20.25%, leading to fell off in CD4+ cells. It was also found that T-suppressors number declined by 14.28%. Ionizing radiation effect led to an increase in the level of DC and MDA, inhibition of the activity of glutathione reductase, glutathione peroxidase and catalase enzymes, as a result of which the oxidative stress formation in the body was observed. Suppression of cellular and humoral immunity, non-specific protection of the body and imbalance of LPO-AOP create preconditions for the emergence of immune pathological state of radiation origin, indicating the necessity to develop promising for adaptation correcting methods.

Keywords: Radiation, Lipid peroxidation, Antioxidant system, Immunity

Introduction

The immune system state plays a pivotal role in determining the body's ability to resist adverse environmental influences. Immune mechanisms are closely linked to other human physiological systems, and their dysfunction inevitably has a detrimental affect the general condition of the body. Additionally, the immune system constitutes one of the main targets for various pathogenic factors (Pennington et al., 2005).

The biological effectiveness of ionizing radiation significantly exceeds the performance of all known modes of radiation (Kuperman, 2018). There is not a single vital function of the body that would not be suppressed by radiation. However, the sensitivity of organs and tissues to ionizing radiation varies, as does their role in radiation pathology and the final consequences of radiation sickness. The most susceptible to radiation are proliferating cells involved in hematopoiesis, while radiation-physiological alterations that disrupt the functioning of hematopoietic organs and blood cells lead to changes in the human immune system. The later of those who live and work in conditions of long-term exposure to radiation of various origins suffers especially (Burlakova, 2004).

Ionizing radiation affect biological systems entails changes in almost all their entities. Immunological reactivity is defined as one of the most radiosensitive functions in both humans and animals. One of the characteristic features of radiation exposure is the long-term preservation of damage in individual components of the immune system, inducing long-term consequences and complications, including the development of malignant neoplasms.

Ionizing radiation can affect not only the differentiation of T-helper cells, but also other components of the immune response. For instance, exposure to ionizing radiation can activate macrophages and dendritic cells, which in turn changes the profile of cytokines released in the tumor microenvironment. This can provoke the antiblastomic immune response suppression and contribute to disease progression. In addition, an imbalance between T-helper subtypes can hinder the formation of an effective immune response memory, which is a critical aspect for achieving long-term outcomes after radiotherapy. Studies show that modification of the immune response towards a more active Th1 response can improve treatment outcomes. Thus, understanding the mechanisms underlying the immune response deteriorations under the influence of ionizing radiation discloses contemporary perspectives for the development of combination therapies that can improve the effectiveness of radiotherapy through immunotherapy or other approaches aimed at restoring the balance between T-helper subtypes. Exposure to ionizing radiation often induces T-helper cell differentiation, resulting in an imbalance of Th1 and Th2 cell subtypes that may cast a shadow over the effectiveness of cancer radiotherapy (Gao et al., 2018).

The biological effects of radiation comprised by atomic and molecular ionization and excitation in organs and tissues, leading to the formation of highly active radicals and peroxides [Shen et al., 2018]. The initial three phases of ionization occur at the molecular level over extremely short periods of time and cause molecular alterations of organs and tissues. In the fourth phase (biological), these alterations are transformed into disturbances in cells, organs, and the body as a whole.

These processes undoubtedly occur at any dose of radiation and can be caused not only by radiation exposure, however also by many other factors not related to radiation (Squillaro et al., 2018). The main pathological conditions, including those induced by radiation, are associated with the active initiation of free radical oxidation (FRO) processes. Currently, the mechanisms of urgent nonspecific adaptation at the cellular and subcellular levels after radiation damage have been studied. At the same time, the violation of metabolic processes regulation in cells can act not only as a consequence, but also as a key element of the pathogenetic mechanisms of radiation damage. Exposure to ionizing radiation is characterized by a noticeable activation of FRO processes. It has been established that ionizing radiation contributes to an increase in the concentration of free radicals in various organs and tissues (Shaban et al., 2017). Recent studies emphasize the essential role of free radical processes in the development of occupational pathology.

Recent studies highlight the significant role of reactive oxygen species (ROS) in the mechanisms of development of pathological damage caused by tissue factors (Pedersen et al., 2016). The interaction of phagocytes with harmful particles promotes increased ROS formation, since such particles are resistant to their effects and constantly activate cells. Excessive ROS production can harm the body's own cells, as well as damage nuclear structures due to oxidative modification of proteins, lipids, and nucleic acids (Serrano-Posada et al., 2015). Activation of phagocytes can spontaneously increase, creating conditions for the formation of a vicious circle of inflammation in the affected tissues. Significant consequences of free radicals' effect on living systems include a mutagenic effect and disruption of the structure and function of cells, which is due to the initiation of lipid peroxidation (LPO) processes (Soodaeva et al., 1982). Under normal physiological conditions, LPO is restrained by the antioxidant system, but its functioning can be disrupted under the influence of unfavorable factors (Simioni et al., 2018).

Taking into consideration the importance of the immune system and metabolic processes in the development of pathological condition, as well as their susceptibility to change, high sensitivity and serious consequences in

disrupted, it seems relevant to study their role in the formation of the pathological process in animals under the influence of high doses of ionizing radiation in experimental conditions.

Study Objectives

The aim of the study was to explore biochemical and immunological indicators in experimentally irradiated animals.

Method

To achieve the stated goal, two experimental cycles were conducted on white male Wistar laboratory rats weighing 200±20 grams, which were kept on a standard diet and in normal vivarium conditions. The first experiment involved 20 intact rats, the second – 20 rats irradiated with a dose of 6 Gy. The experiments with rats were completed by the method of partial decapitation under the influence of light ether anesthesia, 14 days after irradiation. In this case, the requirements set forth in the Helsinki Declaration on the humane treatment of animals were strictly observed (World Medical Association, 2002).

The second group animals were exposed to a single irradiation dose of 6 Gy using ^{60}Co gamma rays on the TERAGAM radiotherapeutic device (ISOTRENDspol. s.r.o., Czech Republic). Before the irradiation procedure, a thorough topometric and dosimetric adjustment was carried out, including placing the object on the isocentric therapeutic table of the Terasix X-ray simulator (Czech Republic), which is similar in its characteristics and purpose to the table of the gamma irradiator. Data on the cross-section of the pattern of irradiated animals, obtained after output to the display screens, were transmitted to the planning system via a computer network using a digitizer. The PlanW-2000 program calculated isodoses, creating a topometric and dosimetric map indicating the technical parameters and planned irradiation doses.

The animals were exposed to total gamma-irradiation at a dose of 6 Gy once: SSD - 97.2 cm, SAD - 100.0 cm, field 40x40 cm, t = 352 sec. (SSD is the distance from the source of ionizing radiation in the apparatus to the conventional center of the irradiated pathological focus; SAD is the distance from the source of ionizing radiation in the apparatus to the closest surface of the irradiated object). During irradiation, the animals were kept in a specially designed cage made of organic glass with isolated cells for each animal.

During the experiment, blood was taken from all animals and the total number of white blood cells (leukocytes) and their subtype, lymphocytes, were counted. For a more detailed analysis of lymphocytes, namely B- and T-lymphocytes and their various subtypes, the immunofluorescence method was used. This method involves staining the cells with special antibodies that "glow" under a fluorescence microscope owing to the fluorochrome attached to them - fluorescein isothiocyanate (FITC). Antibodies that recognize CD3+, CD4+, CD8+ and CD20+ markers on the surface of lymphocytes (these markers indicate belonging to certain subtypes of lymphocytes) were purchased from the American company GALTAG Laboratories. After staining, the cells were examined under a fluorescence microscope, which allowed for a quantitative assessment of the various populations of lymphocytes.

To assess the activity of neutrophils, the nitroblue-tetrazolium test (NBT) was performed and the relative density of insoluble blue formazan granules formed in leukocytes was calculated (Damle et al., 2022). The leukocyte sensitization degree is expressed by the leukocyte migration inhibition reaction with phytohemagglutinin (Artemova, 1973) and the concentration of circulating immune complexes (CIC) (Grinkevich et al., 1981). For quantitative data processing, the immunoregulatory index was determined. In all animals, lipid peroxidation outputs and antioxidant defense enzymes (AOP) were determined in organs and cells. Lymphocytes were isolated from peripheral blood, and homogenates were prepared from the pancreas. The content of diene conjugates (DC) [Gavrilov et al., 1983] and malon dialdehyde (MDA) (Konyuhova et al., 1989), enzyme glutathione reductase (GLR) and glutathione peroxidase (GLP) (Vlasova et al., 1990), catalase (CT) activity (Korolyuk et al., 1988) were determined in them.

The obtained data were subjected to statistical processing using the program "Statgraphics Plus for Windows" (Statpoint Technologies, Inc.). For the analysis, group indicators of summary statistics were calculated, including the arithmetic mean (M) and standard deviation. The reliability of differences was assessed using Student's t-test.

Results and Discussion

The study showed that in experimental animals, exposure to high doses of radiation acted as an immunosuppressive agent, with significant sensitivity of leukocytes and T-lymphocytes and their subpopulations. The number of leukocytes in irradiated animals decreased by 19.0% ($p<0.05$). A decrease in the percentage and absolute number of lymphocytes by 17.62% ($p<0.05$) and 9.05% ($p>0.05$), respectively, was observed (Table 1). As for T-lymphocytes, the following changes were observed: the total number was reduced by approximately 20.25% ($p<0.05$), and the relative number was reduced by 15.36% ($p<0.05$). Accordingly, cells with CD4+ helper cell activity were significantly suppressed: the total number decreased by 21.33% ($p<0.05$), and the relative number decreased by 21.80% ($p<0.05$). The researchers found that the activity on the T-suppressor side was suppressed, with an overall decrease of 14.28% ($p<0.05$), and a relative number of 12.25% ($p>0.05$) (Table 1). Analysis of the study results showed that the absolute and relative number of CD20+ lymphocytes in the blood after irradiation in animals did not change significantly, compared to the control, there was a tendency for these indicators to decrease ($p<0.05$).

It is commonly known that the lymphokine-producing activity of T-lymphocytes reflects the functional activity of the T-system of immunity (Yahyapour et al., 2018). The study showed that irradiated animals showed a decrease in the lymphokine-producing capacity of T-lymphocytes, an increase in the migration index in the leukocyte migration inhibition reaction (LMIC) to PHA by 28.41% compared to the control group ($p<0.05$). In the control group, the ability of cells to produce cytokines that suppress leukocyte migration was expressed. Whereas when exposed to gamma-radiation, cytokine production was apparently significantly lower, thereby exerting an immunosuppressive effect of this factor, which led to the suppression of cytokine synthesis, since the migration index was higher than in intact animals. According to a numerous researcher (Khan et al., 2018, Rak et al., 2015), ionizing radiation causes a decrease in DNA synthesis and the number of cells transformed into blasts under the influence of PHA, a decrease in the production of MIF and the cytotoxic effect. At the same time, ionizing radiation causes a number of interrelated specific and non-specific manifestations. Suppressed CD4+ level and stable CD20+ level indicate the implementation of Th2 immune response, which thereby activates B-cells, evidence of this is the presence of CD20+ concentration at the level of the intact group.

Against the background of suppression of cellular immunity, the result of the immunoregulatory index (IRI) was obtained, a shift to the left towards an insignificant decline of 8.95% ($p> 0.05$). A decrease in IRI is characteristic of immunodeficiency states, oncological diseases. Thus, a pathognomonic laboratory manifestation of immune deficiency is almost complete or progressing to the complete absence of T-helpers and a decrease in the immunoregulatory index. A decrease in the immunoregulatory index in our example occurred as a result of a sharp decrease in T-cells with helper activity compared to T-cells with suppressor activity. T-helpers stimulate the population of B-lymphocytes to the process of antibody production. T-helpers make up approximately 55-60% of the total number of circulating T-lymphocytes. Insufficiency of the helper function of T-lymphocytes leads to a decrease in the body's sensitivity to antigen stimulation, contributing to the development of severe infectious complications, the development of possibly malignant neoplasms of radiation origin. Given the complexity of the genesis of changes in immunity during radiation injury to the body, the task was set to experimentally study the reaction of the non-specific phagocytic link of the body during general gamma-irradiation.

In the blood serum of experimental rats, a decrease in the concentration of circulating immune complexes (CIC) by 20.58% was observed compared to the control values ($p<0.05$). Accounting for oxygen-dependent phagocytic killing in the NBT recovery test is an indicator of the phagocytic and metabolic activity of neutrophil granulocytes. The NBT test reflects the final reaction of one of the key enzyme systems responsible for the effector potential of phagocytes. The study showed that the NBT test indicator in experimentally irradiated animals decreased by 34.65% ($p<0.05$), indicating suppression of the functional activity of neutrophils (Table 1).

Thus, in experimental rats irradiated with gamma-radiation, changes were revealed, characterized, first of all, by a decrease in the absolute and percentage number of T-lymphocytes and their subpopulation (CD4+, CD8+), the functional activity of T-lymphocytes, and the body's defense mechanisms in response to exposure to high doses of radiation. A decrease in the number of CD3+ and their subpopulation, which is the immune system's response to the developing pathological process, on the one hand, can be considered as a general physiological reaction of the body in response to stimulation such as stress (Shen et al., 2018). On the other hand, the presence of deteriorations of qualitative and quantitative indicators of cellular and non-specific phagocytic links of immunity, which is a fact of the development of radiation-induced immunodeficiency. The proliferative activity

of T-cells, the quantitative and functional state of the T-system of immunity also decrease. Analysis of the factual material showed that gamma radiation at a dose of 6 Gy has a suppressive effect not only on the T-cell link, but also on the non-specific phagocytic link of immunity, indicating a decrease in the functional and metabolic activity of neutrophils.

Table 1. The results of the study of the immune system under the influence of radiation

	Control group		Experimental group	
Indicators	Abs. number	%	Abs. number	%
WBC, $\times 10^9$/l	6,26±0,36	-	5,07±0,39 *	-
Lymphocytes, $\times 10^9$/l	2,61±0,16	35,78±2,17	2,15±0,16 *	32,54±2,35
T-lymphocytes (CD3+), $\times 10^9$/l	1,58±0,09	29,55±1,47	1,26±0,06 *	25,01±1,71 *
T-helpers (CD4+), $\times 10^9$/l	0,75±0,05	18,48±1,54	0,59±0,03 *	14,45±1,04 *
T-suppressors (CD8+), $\times 10^9$/l	0,56±0,03	11,43±0,63	0,48±0,02 *	10,03±0,51
B-lymphocytes (CD20+) $\times 10^9$/l	0,43±0,03	6,64±0,48	0,41±0,02	6,08±0,35
IRI (CD4/CD8)	1,34±0,07	-	1,22±0,05	-
LMIR	0,88±0,05	-	1,13±0,08 *	-
Circulating immune complexes (CIC)	1,36±0,08	-	1,08±0,06 *	-
Nitro blue tetrazolium (NBT-test, %)	-	3,78±0,26	-	2,47±0,18 **

Note: The difference is significant compared to the control group * - p<0,05; ** - p<0,01.

At the next stage, the effect of high doses of radiation on the formation of lipid peroxidation products DC and MDA in organs and lymphocytes was studied. The results of the study showed (Table 2) that after irradiation, the concentration of DC in peripheral blood lymphocytes in animals exceeded the control values by 62.07% (p<0.01), and in pancreatic homogenate - by 66.19% (p<0.01). And the concentration of MDA was also recorded with an increase in peripheral blood lymphocytes and pancreatic homogenate of experimental rats: 69.23% (p<0.01) and 57.14% (p<0.01), respectively, compared with the control animals.

The results show that exposure to radiation factors activates the oxidation of free radicals, which may be associated with a decrease in the activity of antioxidant enzymes in the studied objects. Activation of lipid peroxidation is based on the excessive generation of active forms of oxygen, exceeding the physiological capabilities of AOS, which arise after the depletion of enzyme systems, as well as a combination of these mechanisms in the case of exposure to a radiation factor, on the one hand, is determined by the massive death of radiosensitive cells of the body and the loss of antioxidants, and on the other hand, by the generation of an active initiator of LPO (Azzam et al., 2012;Shishkina et al., 2015).

Table 2. The results of the study of LPO of the body under the influence of radiation

LPO output	Research object	Control group	Experimental group
DC	Pancreas	0,71±0,06	1,18±0,10 **
	Blood lymphocytes	0,29±0,02	0,47±0,04 **
MDA	Pancreas	0,21±0,02	0,33±0,02 **
	Blood lymphocytes	0,13±0,01	0,22±0,02 **

Note: The difference is significant compared to the control group ** - p<0,01.

In the next series of experiments, the effect of high doses of ionizing radiation on the antioxidant system of the pancreas and blood lymphocytes was studied: on the activity of the enzymes catalase, glutathione peroxidase and glutathione reductase. One of the enzymes of the antioxidant defense of the body is catalase, which is involved in the removal of active forms of oxygen, thereby increasing the adaptive response of the body. After irradiation, the suppression of catalase activity in the two studied objects - the pancreas and peripheral blood lymphocytes - remains: 13.74% (p>0.05) and 23.57% (p<0.05), respectively (Table 3).

Table 3. The results of the study of the AOP of the body under the influence of radiation

Enzime	Research object	Control group	Experimental group
Glutathione reductase	Pancreas	28,15±2,56	21,34±1,81 *
	Blood lymphocytes	13,14±0,97	8,18±0,63 **
Glutathione peroxidase	Pancreas	158,24±10,17	138,26±12,37
	Blood lymphocytes	422,66±30,42	347,24±21,75*
Catalase	Pancreas	67,63±5,22	58,34±4,36
	Blood lymphocytes	88,22±6,27	67,43±4,15 *

Note: The difference is significant compared to the control group * - p<0,05, ** - p<0,01.

An important enzyme of the antioxidant defense of the body is glutathione peroxidase, which protects the body from oxidative damage of any genesis [Matoušková et al., 2018]. GLP catalyzes the reduction of lipid peroxides to the corresponding alcohols and the reduction of hydrogen peroxide to water. In animals exposed to gamma radiation, glutathione peroxidase activity decreased: in the pancreas - by 12.63% ($p > 0.05$) and in blood lymphocytes - by 17.84% ($p<0.05$). In the reaction of glutathione reductase to irradiation in both studied objects, depression is observed: in the pancreas - 24.19% ($p<0.05$), in lymphocytes - 37.75% ($p<0.01$).

Thus, a sharp decrease in the activity of glutathione reductase, catalase and glutathione peroxidase was detected during radiation exposure of the pancreatic homogenate and blood cells studied. Adaptation processes of the body, as is known, also depend on the function of the AOD system. The experimental radiation pathological process is accompanied by a pronounced disruption of the functional activity of the most crucial adaptive and adaptive systems of the body and the accumulation of toxic compounds in tissues that affect their function. The antioxidant system of the cell, tissue and body as a whole ensures the binding and modification of free radicals, preventing the formation and destruction of biomolecules (Barrera, 2012; Mitciev, 2015).

The results of the studies show significant changes in lipid peroxidation and the antioxidant system during radiation stress. Disruption of the functional connections of the catalytic reduction system of glutathione, accompanied by an inhibitory direction of change in the activity of glutathione-dependent enzymes and long-term stress of the links of the antioxidant system, may contribute to a decrease in the antioxidant status of the body, which indicates the need to develop promising methods of adaptive correction in stress of radiation genesis.

Conclusion

Exposure to high doses of radiation causes suppression of the cellular immune system, especially T-lymphocytes and their subpopulations, and adaptive mechanisms of the body of an immune nature. Exposure to ionizing radiation led to a disruption of antioxidant defense mechanisms and the development of lipid hyperperoxidation syndrome.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflicts of Interest

* The authors declare no conflict of interest.

Funding

* This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant No. AP19677010.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Artemova, A.G. (1973). The phenomenon of the inhibition of migration of blood leukocytes in guinea pigs with delayed hypersensitivity to a foreign tissue agent. *Bjulleten' Jeksperimental'noj Biologii i Mediciny, 10,* 67-71.

Azzam, E. I., Jay-Gerin, J. P., & Pain, D. (2012). Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters*, *327*(1-2), 48-60.

Barrera, G. (2012). Oxidative stress and lipid peroxidation products in cancer progression and therapy. *International Scholarly Research Notices*, *2012*(1), 137289.

Burlakova, E. B. (2004). Life in the atomic and chemical world. *Voprosy Biologicheskoj, Medicinskoj i Farmacevticheskoj Himii*, *4,* 3-4.

Damle, V. G., Wu, K., Arouri, D. J., & Schirhagl, R. (2022). Detecting free radicals post viral infections. *Free Radical Biology and Medicine*, *191*, 8-23.

Gao, H., Dong, Z., Gong, X., Dong, J., Zhang, Y., Wei, W., ... & Jin, S. (2018). Effects of various radiation doses on induced T-helper cell differentiation and related cytokine secretion. *Journal of Radiation Research*, *59*(4), 395-403.

Gavrilov, V. B., & Mishkorudnaya, M. I. (1983). Spectrophotometric determination of the content of lipid hydroperoxides in blood plasma. *Laboratorneo Delo*, *3*, 33-36.

Grinkevich, J. J., Alferov A.N. (1981). Determination of immune complexes in the blood of cancer patients. *Laboratornoe Delo, 8,* 493-495.

Khan, K., Tewari, S., Awasthi, N., Mishra, S.P., Agarwal, G.R., Rastogi, M. & Husain, N. (2018). Flow cytometric detection of gamma-H2AX to evaluate DNA damage by low dose diagnostic irradiation. *Med Hypotheses,115,* 22-28.

Korolyuk M.A., Ivanova L.I., Majorova N.O., & Tokarev, V. E. (1998). Method for determination of catalase activity. *Laboratornoe Delo, 1*, 16-19.

Kuperman, V. Y. (2018). Effect of radiation protraction in hypofractionated radiotherapy. *Medical Physics*, *45*(7), 3442-3448.

Matoušková, P., Hanousková, B., & Skálová, L. (2018). MicroRNAs as potential regulators of glutathione peroxidases expression and their role in obesity and related pathologies. *International Journal of Molecular Sciences*, *19*(4), 1199.

Mitciev, A.K. (2015). Change in the activity of lipid peroxidation as the mechanism of kidney pathology development under upon heavy metals action. *Patologicheskaya Fiziologiya i Eksperimental'naya Terapiya, 2,* 65-69.

Pedersen, J. T., Chen, S. W., Borg, C. B., Ness, S., Bahl, J. M., Heegaard, N. H., ... & Teilum, K. (2016). Amyloid-β and α-synuclein decrease the level of metal-catalyzed reactive oxygen species by radical scavenging and redox silencing. *Journal of the American Chemical Society*, *138*(12), 3966-3969.

Pennington, D. J., Vermijlen, D., Wise, E. L., Clarke, S. L., Tigelaar, R. E., & Hayday, A. C. (2005). The integration of conventional and unconventional T cells that characterizes cell-mediated responses. *Advances in Immunology*, *87*, 27-59.

Rak, J., Chomicz, L., Wiczk, J., Westphal, K., Zdrowowicz, M., Wityk, P., ... & Golon, L. (2015). Mechanisms of damage to DNA labeled with electrophilic nucleobases induced by ionizing or UV radiation. *The Journal of Physical Chemistry B*, *119*(26), 8227-8238.

Serrano-Posada, H., Centeno-Leija, S., Rojas-Trejo, S. P., Rodríguez-Almazán, C., Stojanoff, V., & Rudiño-Piñera, E. (2015). X-ray-induced catalytic active-site reduction of a multicopper oxidase: structural insights into the proton-relay mechanism and O2-reduction states. *Biological Crystallography*, *71*(12), 2396-2411.

Shaban, N. Z., Ahmed Zahran, A. M., El-Rashidy, F. H., & Abdo Kodous, A. S. (2017). Protective role of hesperidin against γ-radiation-induced oxidative stress and apoptosis in rat testis. *Journal of Biological Research-Thessaloniki*, *24*, 1-11.

Shen, Y., Jiang, X., Meng, L., Xia, C., Zhang, L., & Xin, Y. (2018). Transplantation of bone marrow mesenchymal stem cells prevents radiation-induced artery injury by suppressing oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*, *2018*(1), 5942916.

Shishkina L.N., Zagorskaya N.G., & Shevchenko O.G. (2015). The role of antioxidant tissue status in the mouse's response to chronic exposure in early ontogeny. *Radiacionnaya Biologiya*,*21*, 91-6.

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget*, *9*(24), 17181.

Soodaeva, S.K., Skotzelias, E.D., Zhukov, A.A., & Archakov, A.I. (1982). Comparative studies of superoxide generation in microsomes and reconstituted monooxygonose systems. In *Cytochrome P-450. biochemistry, biophysies and environmental implications* (pp.615-618). Amsterdam: Biomedicine Press.

Squillaro, T., Galano, G., De Rosa, R., Peluso, G., & Galderisi, U. (2018). Concise review: the effect of low-dose ionizing radiation on stem cell biology: A contribution to radiation risk. *Stem Cells*, *36*(8), 1146-1153.

Vlasova S.N., Shabunina E.I., & Pereslegina, I.A. (1990). The activity of glutathione-dependent enzymes of erythrocytes in chronic liver diseases in children. *Laboratornoe Delo, 8*,19-22.

World Medical Association. (2002). *Declaration of Helsinki. Ethical Principles for Medical Research Involving Human participants* (pp. 42-46). Retrieved from https://www.wma.net/

Yahyapour, R., Amini, P., Rezapour, S., Cheki, M., Rezaeyan, A., Farhood, B., ... & Najafi, M. (2018). Radiation-induced inflammation and autoimmune diseases. *Military Medical Research*, *5*, 1-8.

Author(s) Information

Oralbek Ilderbayev
L.N. Gumilyov Eurasian National University
010008 Astana, Satpayev 2, Republic of Kazakhstan.
Contact e-mail: *oiz5@yandex.ru*

Kerim Mutig
I.M. Sechenov First Moscow State Medical University,
119991, 8-2 Trubetskaya str. Moscow, Russian Federation

Gulzhan Ilderbayeva
L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2, Republic of Kazakhstan

Darkhan Uzbekov
L.N. Gumilyov Eurasian National University
010008 Astana, Satpayev 2, Republic of Kazakhstan.

Aruzhan Ilderbayeva
University of Duisburg-Essen,
45117 Essen, Germany

Ainur Zhanilova
L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2, Republic of Kazakhstan

To cite this article:

Ilderbayev, O., Mutig, K., Ilderbayeva, G., Uzbekov, D., Ilderbayeva, A., & Zhanilova, A. (2025). Acute ^{60}Co Γ-irradiation effect on lipid peroxidation and immune system in experimental animals. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 27-34.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 35-38

ICGeHeS 2025: International Conference on General Health Sciences

Sustainability and Organic Livestock in 2050: Challenges and Innovations

Muhammad Safdar
Cholistan University of Veterinary and Animal Sciences

Mehmet Ozaslan
Gaziantep University

Abstract: The global demand for sustainable and organic livestock farming is expected to rise significantly by 2050 due to increasing concerns over climate change, food security, and ethical animal husbandry. Organic livestock production focuses on environmentally friendly practices, reduced reliance on synthetic inputs, and improved animal welfare. Advancements in regenerative agriculture, precision farming, and circular economy models will drive innovation in organic livestock systems. The integration of artificial intelligence, smart sensors, and sustainable feed alternatives will optimize resource efficiency while maintaining organic standards. Additionally, genetic advancements and microbiome research will enhance disease resistance and animal productivity in organic farming systems. However, challenges such as land use limitations, high production costs, disease management, and market accessibility remain key hurdles. Policy reforms, consumer awareness, and technological innovations will be crucial in shaping the future of organic livestock. A holistic approach integrating sustainability, economic feasibility, and ethical considerations will ensure a resilient and efficient organic livestock industry by 2050.

Keywords: Sustainability, Organic, Livestock, Innovation

Introduction

Organic livestock farming aligns with the United Nations' Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger) and SDG 13 (Climate Action), by promoting sustainable production and environmental stewardship. By 2050, global meat and dairy demand is expected to increase by 68% (Alexandratos & Bruinsma, 2012), challenging organic systems to scale without compromising their principles. Constraints include limited arable land, organic feed scarcity, high emissions intensity, and economic barriers. However, innovations like precision technologies, novel feeds, and circular farming offer solutions. This revised paper incorporates recent studies (2020-2025) to provide an updated, original perspective on the future of organic livestock farming.

Challenges in Organic Livestock Farming

Land Scarcity and Resource Competition

Organic livestock systems, reliant on pasture-based grazing and organic crop integration, require more land due to lower yields, typically 19-25% below conventional systems (Seufert et al., 2012). By 2050, arable land availability will shrink due to urbanization, soil degradation, and climate change, with a projected 12% reduction in suitable agricultural land in tropical regions (Cai & Zhang, 2011). Competition from bioenergy and human food crops exacerbates this issue. Recent studies highlight that land use conflicts could intensify, with organic systems needing innovative land management to remain viable (Smith et al., 2022).

Organic Feed Shortages

Organic livestock depend on certified organic feed, free from synthetic fertilizers and GMOs, which is land- and water-intensive to produce. Feed accounts for 60-70% of livestock's environmental footprint, with organic feed amplifying resource demands (Mottet et al., 2017). Climate variability may reduce grain yields by 10-15% by 2050, increasing competition between human and animal consumption (Wheeler & von Braun, 2013). Recent research underscores the rising cost of organic feed, which limits scalability, particularly for small-scale farmers (Willer et al., 2023).

Greenhouse Gas Emissions

Organic livestock contribute to global GHG emissions through methane from ruminants and nitrous oxide from manure. Due to lower productivity, organic systems can emit up to 20% more GHGs per kilogram of beef than conventional systems (Meier et al., 2015). Achieving net-zero emissions by 2050, a global priority, is challenging for organic systems reliant on extensive grazing. Recent analyses confirm that methane emissions remain a critical hurdle, necessitating targeted mitigation strategies (Jones et al., 2021).

Economic and Market Barriers

Higher costs from certification, labor, and premium feed reduce the economic viability of organic livestock farming. Smallholders, particularly in developing countries, face barriers to certification and market access, limiting scalability (Jouzi et al., 2017). Recent data indicate that market saturation and volatile consumer demand for organic products could further strain profitability by 2050 (Reganold & Wachter, 2020). Subsidies favoring conventional agriculture continue to disadvantage organic producers, requiring policy reform.

Innovations for Sustainable Organic Livestock in 2050

Precision Livestock Farming

Precision livestock farming (PLF) uses IoT, AI, and sensors to optimize resource use and animal welfare. Methane-monitoring devices can reduce emissions by 15-20% through dietary adjustments (Hammond et al., 2016). Drones and satellite imagery enhance pasture management, reducing soil degradation. Recent advancements in PLF, including AI-driven health monitoring, promise to align with organic standards while boosting efficiency (Banhazi et al., 2024). By 2050, PLF could be ubiquitous in organic systems, with blockchain ensuring traceability.

Novel Feed Sources

Alternative feeds like insect meal and microalgae address feed scarcity sustainably. Black soldier fly larvae, requiring 70% less land than soy, can utilize organic waste, aligning with organic principles (Makkar et al., 2014). Microalgae, cultivated in bioreactors, offer high-protein feed with minimal land use. Recent studies project that by 2050, insect and algae-based feeds could supply 20-30% of organic livestock diets, reducing environmental impacts (Parodi et al., 2022).

Circular Farming Systems

Circular agriculture recycles nutrients and energy, enhancing sustainability. Anaerobic digesters convert manure into biogas, offsetting 50-60% of farm energy needs, while digestate fertilizes crops (Holm-Nielsen et al., 2009). Silvopastoral systems, integrating trees with grazing, sequester carbon and improve soil health. Recent research emphasizes the scalability of circular systems, with policy incentives driving adoption (Dumont et al., 2023). By 2050, circular models could dominate organic livestock farming.

Policy and Market Reforms

Policies such as carbon pricing and organic subsidies can bolster sustainability. True-cost accounting, reflecting environmental externalities, could enhance organic competitiveness (Sandhu et al., 2015). Harmonized global certification standards would ease market access for smallholders. Recent policy analyses advocate for consumer education to sustain demand for organic products (Willer et al., 2023). By 2050, integrated policy frameworks could position organic livestock as a pillar of sustainable food systems.

Discussion

Organic livestock farming in 2050 must balance productivity with environmental and social goals. Innovations like PLF and novel feeds improve efficiency but require investment and training, particularly in developing regions. Circular systems offer long-term benefits but demand infrastructure. Recent studies highlight the need for equitable technology access to support smallholders, who produce 70% of organic livestock in developing nations (Willer et al., 2023).

Original projections estimate that organic livestock could meet 12-18% of global meat and dairy demand by 2050 if innovations are scaled. Methane mitigation, such as seaweed-based feed additives reducing emissions by up to 80% (Roque et al., 2021), is critical. Agroecological practices could narrow the yield gap with conventional systems by 50%, enhancing land efficiency. Policy reforms must prioritize inclusivity to ensure smallholders benefit from these advancements.

Conclusion

Organic livestock farming in 2050 can significantly contribute to sustainable food systems if challenges are addressed through innovation and policy. Land scarcity, feed shortages, emissions, and economic barriers are formidable but surmountable with precision farming, novel feeds, circular systems, and market reforms. Recent research underscores the urgency of scaling these solutions equitably. Continued investment, research, and collaboration will be vital to realizing organic livestock's potential in a sustainable future.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* There is no fund for this article.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Alexandratos, N., & Bruinsma, J. (2012). *World agriculture towards 2030/2050: The 2012 revision*. FAO. Retrieved from http://www.fao.org/3/ap106e/ap106e.pdf

Banhazi, T. M., Berckmans, D., & Halachmi, I. (2024). Precision livestock farming: Opportunities and challenges for sustainable animal production. *Animal Frontiers, 14*(1), 12-20.

Dumont, B., Ryschawy, J., & Duru, M. (2023). Circular agriculture: A pathway for sustainable livestock systems. *Agricultural Systems, 205*, 103578.

Hammond, K. J., Crompton, L. A., Bannink, A., Dijkstra, J., Yáñez-Ruiz, D. R., O'Kiely, P., Kebreab, E., Eugène, M. A., Yu, Z., Shingfield, K. J., Schwarm, A., Hristov, A. N., & Reynolds, C. K. (2016). Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. *Animal Feed Science and Technology, 219*, 13-30.

Holm-Nielsen, J. B., Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilization. *Bioresource Technology, 100*(22), 5478-5484.

Jones, A. K., Jones, D. L., & Cross, P. (2021). Methane emissions from organic livestock systems: Challenges and mitigation strategies. *Journal of Environmental Management, 295*, 113098.

Jouzi, Z., Azadi, H., Taheri, F., Zarafshani, K., Gebrehiwot, K., Van Passel, S., & Lebailly, P. (2017). Organic farming and small-scale farmers: Main opportunities and challenges. *Ecological Economics, 132*, 144-154.

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology, 197*, 1-33.

Meier, M. S., Stoessel, F., Jungbluth, N., Juraske, R., Schader, C., & Stolze, M. (2015). Environmental impacts of organic and conventional agricultural products—Are the differences so clear? *Journal of Cleaner Production, 99*, 1-13.

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C., & Gerber, P. (2017). Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. *Global Food Security, 14*, 1-8.

Parodi, A., Leip, A., De Boer, I. J. M., & Ziegler, F. (2022). Alternative protein sources for sustainable livestock production: A review. *Frontiers in Sustainable Food Systems, 6*, 821456.

Reganold, J. P., & Wachter, J. M. (2020). Organic agriculture in the twenty-first century: Challenges and opportunities. *Nature Plants, 6*(2), 76-84.

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS ONE, 16*(3), e0247820.

Sandhu, H. S., Wratten, S. D., & Cullen, R. (2015). The role of ecosystem services in organic farming: An overview. In *Organic farming for sustainable agriculture* (pp. 1-18). Springer.

Seufert, V., Ramankutty, N., & Foley, J. A. (2012). Comparing the yields of organic and conventional agriculture. *Nature, 485*(7397), 229-232.

Smith, L. G., Kirk, G. J. D., Jones, P. J., & Williams, A. G. (2022). The greenhouse gas impacts of converting food production in England and Wales to organic methods. *Nature Communications, 13*(1), 2871.

Wheeler, T., & von Braun, J. (2013). Climate change impacts on global food security. *Science, 341*(6145), 508-513.

Willer, H., Schlatter, B., & Trávníček, J. (2023). *The world of organic agriculture: Statistics and emerging trends 2023*. Retrieved from https://www.organic-world.net/yearbook/yearbook-2023.html

Zhang, W., & Cai, X. (2011). Climate change impacts on global agricultural land availability. *Environmental Research Letters, 6*(1), 014014.

Author(s) Information

Muhammad Safdar
Cholistan University of Veterinary and Animal Sciences
Bahawalpur, Pakistan
Contact e-mail: *msafdar@cuvas.edu.pk*

Mehmet Ozaslan
Gaziantep University
Gaziantep, Türkiye

To cite this article:

Safdar, M., & Ozaslan, M. (2025). Sustainability and organic livestock in 2050: Challenges and innovations. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 35-38.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 39-46

ICGeHeS 2025: International Conference on General Health Sciences

Evaluating the Role of ChatGPT in Health Information Provision: Capabilities, Limitations, and Ethical Implications

Rumyana Stoyanova
Medical University Plovdiv

Aleksandar Stoyanov
Technical University of Sofia

Abstract: This study provides a critical analysis of ChatGPT's strengths and weaknesses as a resource for delivering health-related information, emphasizing its potential for both general advice and tailored health guidance. Through a systematic review and expert analysis, the study highlights ChatGPT's ability to deliver immediate and accessible information on a wide range of health topics, including nutrition and chronic disease management. While its conversational interface and capacity for personalization make it a valuable resource for users seeking initial advice, significant limitations are evident in its handling of complex and nuanced health scenarios. These shortcomings are primarily attributed to gaps in its training, including outdated data and potential incorporation of unverified sources. The findings emphasize the importance of recognizing ChatGPT as a supplementary tool rather than a replacement for professional healthcare consultation. Ensuring user safety requires ongoing updates to its training datasets, integration of the latest scientific evidence, and the establishment of clear guidelines for its application in healthcare settings. The study underscores the critical role of qualified professionals in verifying and contextualizing AI-generated advice, particularly in complex or high-risk cases. Future research and development are essential to enhance ChatGPT's reliability, accuracy, and effectiveness, ensuring its optimal contribution to health information dissemination while maintaining the highest standards of safety and ethics.

Keywords: Health information, ChatGPT, Professional healthcare, Patient safety

Introduction

In recent years, advancements in artificial intelligence (AI) have revolutionized the accessibility and delivery of health-related information. Modern technologies now provide users with instant access to comprehensive resources, aiding in disease prevention, symptom identification, and overall wellness. Among these innovations is ChatGPT, an AI-powered conversational agent developed by OpenAI. ChatGPT utilizes advanced machine learning algorithms to generate relevant and personalized responses based on user input, simulating human-like interactions through text-based communication (Introducing ChatGPT, 2022).

This technology has gained significant attention for its ability to provide general health guidance, making information more accessible to a global audience. However, despite its potential benefits, ChatGPT has notable limitations that must be considered when evaluating its reliability as a health resource.

Strengths of ChatGPT in Health Information Provision

One of the key advantages of ChatGPT is its ability to provide round-the-clock access to general health information. Unlike traditional healthcare systems that require appointments and waiting times, ChatGPT offers

immediate responses to user queries. This feature is particularly beneficial for individuals facing barriers to healthcare access, such as those living in remote areas, those with time constraints, or those who feel hesitant to seek professional medical advice due to personal concerns (Morita et al., 2023; Li et al., 2023).

Additionally, ChatGPT serves as an introductory resource for those seeking foundational knowledge on common illnesses, preventive healthcare practices, and lifestyle recommendations. By providing easily digestible information, it empowers users to make informed decisions about their health and wellness. This is particularly valuable in public health education, where AI-driven chatbots can assist in disseminating information on topics such as vaccinations, nutrition, mental health, and chronic disease management (Chatelan et al. 2023).

Another major strength of ChatGPT is its interactive and user-friendly design. The AI's ability to process natural language inputs and deliver personalized responses makes it a highly accessible tool for diverse populations. Users can engage with the chatbot in multiple languages and across various platforms, ensuring a broad reach. The convenience of having free, on-demand health guidance also enhances its appeal, especially for those seeking preliminary insights before consulting a medical professional (Ponzo et al., 2024).

Weaknesses and Limitations of ChatGPT in Healthcare Contexts

Despite its advantages, ChatGPT has inherent limitations that prevent it from serving as a reliable standalone healthcare solution. First and foremost, AI lacks the ability to conduct real-time medical assessments. Unlike physicians and specialists, ChatGPT cannot analyze vital signs, perform physical examinations, or access patient medical histories. This makes it unsuitable for diagnosing conditions or offering personalized medical treatment plans (Chatelan et al. 2023; Wang et al., 2023; Tripathi & Chandra, 2023).

Moreover, the reliability of ChatGPT's health-related responses is a significant concern. While the AI is trained on extensive datasets, it has a knowledge cutoff, meaning it may provide outdated, incomplete, or even inaccurate information. This is a critical flaw in medical contexts, where precision and up-to-date knowledge are essential. The risk of misinformation can have serious consequences, particularly for individuals seeking urgent medical advice or guidance on managing chronic conditions (Safranek et al., 2023; Liaw et al., 2023).

Linguistic ambiguities further compound the problem. Since ChatGPT relies on interpreting user queries through natural language processing, it may misinterpret vague or poorly structured questions. This can lead to misleading recommendations, which, in a healthcare setting, could result in inappropriate self-treatment or unnecessary anxiety for the user (Morita et al., 2023). Additionally, AI-generated content does not always distinguish between general advice and case-specific medical recommendations, creating potential risks for those who rely on the chatbot for critical health decisions.

Privacy concerns arise when using ChatGPT for health-related inquiries, particularly in terms of data security and confidentiality. While OpenAI has implemented safeguards, ChatGPT does not encrypt conversations end-to-end, meaning that sensitive health information shared by users could be stored or accessed under certain conditions. Additionally, since the AI lacks the ability to differentiate between general queries and personally identifiable medical details, users may unknowingly disclose private health information without proper protection in place. This highlights the need for caution when discussing personal medical history or conditions via AI-driven tools. Users may not always be aware of how their data is handled, raising ethical concerns regarding confidentiality and data security (Thirunavukarasu et al., 2023; Komorowski et al., 2023; Arslan, 2023). This highlights the importance of ensuring AI compliance with stringent health data protection regulations.

Risk Mitigation and the Need for Caution

To mitigate some of these risks, ChatGPT explicitly states that its health-related responses should not be used as a substitute for professional medical advice. In this regard, a study found that almost half of the available online health information related to nutrition was either inaccurate - 48.9%, or of low quality - 48.8% (Denniss et al., 2023). This highlights the need for users to be cautious when relying on AI- driven tools for medical guidance. Healthcare professionals and regulatory bodies must also play a role in guiding the responsible use of AI in health communication.

Study Aim

Given the growing reliance on AI for health information, this systematic review critically assesses ChatGPT's effectiveness, safety, and reliability in delivering health-related advice. To ensure specificity and avoid dilution of the analysis, this study focuses exclusively on ChatGPT's role in providing safe health information related to nutrition and dietary regimens for individuals with chronic diseases. By analyzing its strengths and limitations within this context, this review aims to offer insights into how AI-powered chatbots can complement, rather than replace, professional healthcare services, ensuring that users receive accurate and responsible nutrition-related health information.

Materials and Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological rigor, transparency, and reproducibility (Page et al., 2021).

Study Selection

A comprehensive literature search was conducted in the Scopus and Web of Science databases during January–February 2025. The search strategy employed keywords such as "*ChatGPT* AND *nutrition*" and subsequent terms including "*health*" to identify studies evaluating ChatGPT's capabilities and limitations in generating health-related nutritional information for diverse health conditions (accounting for health status, preferences, and goals). Initial research identified 30 full-text articles and 10 conference reports in Scopus, and 71 full-text articles on the Web of Science.

Eligibility Criteria

Inclusion criteria:

1. Open-access, full-text publications (articles or conference reports).
2. Studies focused on ChatGPT's application in nutrition, dietary regimens, or health-related information generation.
3. Publications addressing contextual factors such as health conditions, user preferences, or clinical goals.

Exclusion criteria:

1. Duplicate publications.
2. Non-open-access articles.
3. Conference abstracts, short communications, or non-peer-reviewed materials.
4. Studies unrelated to ChatGPT's role in nutrition or health contexts.

Data Analysis

The selection process involved three stages:

1. **Duplicate removal**: Cross-referencing results from Scopus and Web of Science revealed significant overlap. After removing duplicates, 55 publications remained.

2. **Open-access screening**: Only 32 of the 55 publications were freely accessible and underwent content analysis.

3. **Content screening**: A final subset of 8 articles directly aligned with the study's objectives (see Figure 1). These were critically analyzed to evaluate ChatGPT's strengths, limitations, and applicability in generating nutrition-related health information.

This structured approach ensured a rigorous, unbiased synthesis of evidence on ChatGPT's role in nutrition and health contexts.

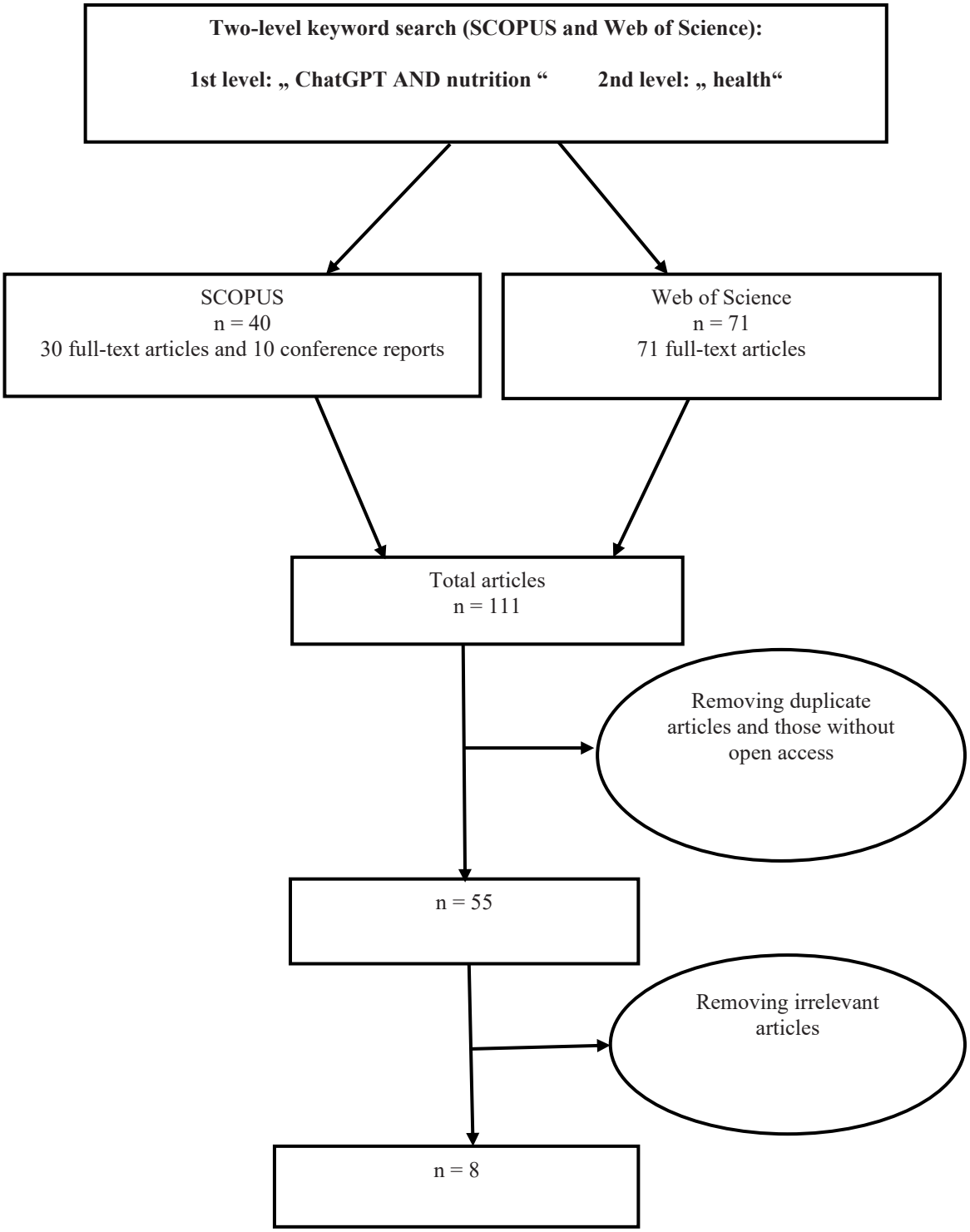

Figure 1. Stages of scientific publication selection.

The diagram illustrates the screening process, including duplicate removal, open-access filtering, and relevance-based exclusion of articles.

Results and Discussion

The studies included in the analysis showed quite contradictory results. Table 1 summarizes the main findings from them.

Table 1. Synthesized summary of studies on ChatGPT's role in dietary and health information

Study	Objective	Key Findings	Conclusion
Chatelan et al. 2023	Assess ChatGPT's impact on dietitians' work and risks of AI-generated advice.	- **Accuracy**: Provides dietary advice for conditions like type 2 diabetes but with inconsistencies and incomplete plans. - **Hallucinations**: Generates plausible but factually incorrect responses. - **Professional impact**: Risks reduced patient interaction and reliance on unqualified advice.	ChatGPT has potential to support dietitians but requires oversight due to risks of misinformation and reduced patient engagement.
Kirk et al. 2023	Compare ChatGPT's answers to dietitians' responses to common dietary queries.	- ChatGPT outperformed dietitians in **scientific correctness** (5/8 questions), **clarity** (5/8), and **practicality** (4/8) in general dietary advice.	ChatGPT matches or exceeds dietitians in answering common dietary questions but lacks nuanced clinical judgment.
Sun et al. 2023	Test ChatGPT's ability to pass China's registered dietitian exam.	- Accuracy: 60.5% (ChatGPT) vs. 74.5% (GPT-4). - Overlap with expert recommendations: 80.7% (non-recommended foods), 94.9% (recommended foods).	Limited use recommended; AI should supplement, not replace, expert-validated dietary advice.
Ponzo et al. 2024	Evaluate ChatGPT's dietary advice for non-communicable diseases (NCDs).	- **Accuracy**: 55.6–73.3% "appropriate" advice across conditions (e.g., hypertension, obesity). - Errors in complex cases (e.g., omega-3 fatty acids recommendations for liver disease).	Effective for general NCD advice but struggles with personalized strategies, necessitating expert consultation.
Papastratis et al. 2024	Compare AI-generated meal plans with knowledge-based systems.	- **Caloric accuracy**: ChatGPT had >19% deviation vs. 0.8% for expert systems. - **Diversity**: ChatGPT-3.5 offered the most varied meal plans (6.58 vs. 4.89).	ChatGPT shows promise for diverse meal planning but requires refinement for clinical accuracy.
Kim et al. 2024	Assess AI-generated weight-loss plans vs. clinical protocols.	- **Safety**: Highest-rated aspect (6.53/10). - Experts could not distinguish AI vs. human plans in blind evaluation.	AI-generated plans are promising but need validation before clinical adoption.
Liao et al. 2024	Evaluate ChatGPT's dietary advice for students' nutritional literacy.	- **Readability**: High scores. - **Completeness**: Lacking in practical advice. - **Accuracy**: 84.38% in literacy tests.	Suitable as an educational tool but requires improvements in practical applicability and completeness.
Papastratis et al. 2024	Develop an AI system for personalized weekly meal plans.	- **Accuracy**: 87% (virtual profiles), 84.19% (real profiles). - Effectively combines user data (health, activity) for tailored plans.	AI-based systems outperform traditional models, offering balanced and diverse meal plans aligned with expert guidelines.

The integration of artificial intelligence (AI) in dietary guidance has shown promising yet inconsistent results. ChatGPT, as a widely used AI tool, demonstrates capabilities in generating general dietary recommendations but falls short in providing precise, individualized advice for complex medical conditions. Several studies highlight both the strengths and limitations of AI-generated dietary recommendations, emphasizing the need for human oversight.

One of the key findings is that ChatGPT has demonstrated strong performance in answering common dietary questions, often matching or exceeding dietitians in scientific accuracy, clarity, and practicality. However, its

effectiveness diminishes when applied to complex medical cases requiring individualized dietary planning (Kirk et al., 2023).

The accuracy of AI-generated dietary advice varies significantly depending on the complexity of the condition. Ponzo et al. (2024) reported that ChatGPT provided appropriate nutritional advice in 55.6–73.3% of cases related to non-communicable diseases (NCDs) such as hypertension and obesity. However, errors arose in more complex cases, such as recommending omega-3 fatty acids for liver disease without considering specific patient requirements. Similarly, Chatelan et al. (2023) identified risks of misinformation, as ChatGPT occasionally generated plausible but factually incorrect responses, reinforcing concerns regarding its reliability.

When evaluating AI-generated meal plans, studies indicate that ChatGPT excels in diversity but struggles with caloric accuracy. Papastratis et al. (2024) found that ChatGPT-created meal plans had a deviation of over 19% in caloric content compared to expert-developed systems, which exhibited only a 0.8% deviation. Although the AI demonstrated strong capabilities in meal variety (6.58 vs. 4.89 in meal diversity scores), its inconsistency in precise nutritional balance highlights the necessity for refinement.

The application of AI in educational contexts appears more promising. Liao et al. (2024) assessed ChatGPT's ability to enhance nutritional literacy and found that its responses scored highly in readability (84.38%), although practical guidance remained insufficient. This suggests that while ChatGPT can serve as an effective tool for general education, its use in clinical dietary planning requires further validation.

A particularly concerning limitation is the AI's inability to differentiate between appropriate and inappropriate food recommendations in specific medical cases. Studies have shown that ChatGPT sometimes includes problematic food choices in dietary plans, such as recommending almond milk for individuals with nut allergies (Niszczota & Rybicka, 2023). Similarly, Chatelan et al. (2023) found that ChatGPT-generated menus for patients undergoing hemodialysis overlapped with those designed for type 2 diabetes, failing to recognize the critical dietary differences between these conditions.

On the other hand, AI-driven meal planning systems that integrate real patient data show greater promise. Papastratis et al. (2024) developed an AI-based system that achieved 87% accuracy in tailoring meal plans to virtual profiles and 84.19% accuracy for real users. This suggests that when AI is combined with structured user data, it can produce more reliable and personalized dietary guidance.

Despite these advancements, ChatGPT's role in nutrition remains supplementary rather than leading. This technology is best suited for initial dietary guidance and educational purposes, while expert consultation remains essential for personalized nutritional planning. Kim et al. (2024) noted, AI-generated weight-loss plans were highly rated for safety (6.53/10), yet they still require clinical validation before being fully integrated into healthcare practice.

Overall, the evidence suggests that ChatGPT is a valuable tool for generating general dietary recommendations, but it cannot be a substitute for trained dietitians. Patients should remain aware of AI limitations, particularly their accuracy inconsistencies in complex cases. Future AI advancements should focus on improving contextual understanding, refining dietary recommendations for specific medical conditions, and ensuring compliance with expert guidelines to maximize its effectiveness in the healthcare sector.

Conclusion

Artificial intelligence, particularly ChatGPT, has transformed the accessibility of health-related information, making it easier for individuals to obtain guidance on general wellness, nutrition, and disease prevention. This technology has the potential to bridge gaps in healthcare access by offering immediate responses, enhancing public health education, and supporting decision-making for those seeking initial health insights. However, despite these benefits, AI-driven tools remain far from replacing human expertise in medical and nutritional fields.

One of the most pressing challenges of AI in healthcare is its inability to interpret real-time patient data, assess individual medical histories, or engage in nuanced decision-making. While AI can provide general guidance, it lacks the critical thinking, experience, and adaptability that human professionals bring to complex cases. The potential for misinformation, inaccuracies, and misinterpretation of user queries further limits its reliability, particularly in sensitive medical contexts.

Beyond accuracy concerns, ethical considerations such as data privacy and patient safety must be carefully managed. AI-generated recommendations should not be blindly trusted, especially when they involve medical conditions that require personalized treatment. Instead, these technologies should be viewed as supplementary tools that enhance, rather than replace, professional healthcare services.

The future of AI in healthcare will depend on improvements in its ability to provide accurate, personalized, and contextually aware recommendations. Collaboration between AI developers, healthcare professionals, and regulatory bodies will be essential to ensure that these tools are used safely and effectively. While AI will continue to play an important role in public health education, disease prevention, and nutritional guidance, human expertise will remain irreplaceable in delivering high-quality, personalized healthcare.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* There is no fund for this article

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Arslan, S. (2023). Exploring the potential of ChatGPT in personalized obesity treatment. *Annals of Biomedical Engineering, 51*(9), 1887–1888.

Chatelan, A., Clerc, A., & Fonta, P. A. (2023). ChatGPT and future artificial intelligence chatbots: What may be the influence on credentialed nutrition and dietetics practitioners? *Journal of the Academy of Nutrition and Dietetics, 123*(11), 1525–1531.

Denniss, E., Lindberg, R., & McNaughton, S. A. (2023). Quality and accuracy of online nutrition-related information: A systematic review of content analysis studies. *Public Health Nutrition, 26*(7), 1345–1357.

Georgadarellis, G. L., Cobb, T., Vital, C. J., & Sup, I. V. F. C. (2024). Nursing perceptions of robotic technology in healthcare: A pretest–posttest survey analysis using an educational video. *IISE Transactions on Occupational Ergonomics and Human Factors, 12*(1–2), 68–83.

Kirk, D., van Eijnatten, E., & Camps, G. (2023). Comparison of answers between ChatGPT and human dieticians to common nutrition questions. *Journal of Nutrition and Metabolism, 2023*(1), 5548684.

Komorowski, M., del Pilar Arias López, M., & Chang, A. C. (2023). How could ChatGPT impact my practice as an intensivist? An overview of potential applications, risks, and limitations. *Intensive Care Medicine, 49*(7), 844–847.

Li, R., Kumar, A., & Chen, J. H. (2023). How chatbots and large language model artificial intelligence systems will reshape modern medicine: Fountain of creativity or Pandora's box? *JAMA Internal Medicine, 183*(6), 596–597.

Liao, L. L., Chang, L. C., & Lai, I. J. (2024). Assessing the quality of ChatGPT's dietary advice for college students from dietitians' perspectives. *Nutrients, 16*(12), 1939.

Liaw, W., Chavez, S., Pham, C., Tehami, S., & Govender, R. (2023). The hazards of using ChatGPT: A call to action for medical education researchers. *Primer, 7*, 27.

Morita, P. P., Abhari, S., Kaur, J., Lotto, M., Miranda, P. A. D. S. E. S., & Oetomo, A. (2023). Applying ChatGPT in public health: A SWOT and PESTLE analysis. *Frontiers in Public Health, 11*, 1225861.

Niszczota, P., & Rybicka, I. (2023). The credibility of dietary advice formulated by ChatGPT: Robo-diets for people with food allergies. *Nutrition, 112*, 112076.

OpenAI. (2023). *Introducing ChatGPT*. Retrieved from https://openai.com/blog/chatgpt

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A.,…& Brennan, S. E. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ, 372*, 71.

Papastratis, I., Konstantinidis, D., Daras, P., & Dimitropoulos, K. (2024). AI nutrition recommendation using a deep generative model and ChatGPT. *Scientific Reports, 14*(1), 14620.

Papastratis, I., Stergioulas, A., Konstantinidis, D., Daras, P., & Dimitropoulos, K. (2024). Can ChatGPT provide appropriate meal plans for NCD patients? *Nutrition, 121*, 112291.

Ponzo, V., Goitre, I., Favaro, E., Merlo, F. D., Mancino, M. V., Riso, S., & Bo, S. (2024). Is ChatGPT an effective tool for providing dietary advice? *Nutrients, 16*(4), 469.

Safranek, C. W., Sidamon-Eristoff, A. E., Gilson, A., & Chartash, D. (2023). The role of large language models in medical education: Applications and implications. *JMIR Medical Education, 9*, e50945.

Sun, H., Zhang, K., Lan, W., Gu, Q., Jiang, G., Yang, X., & Han, D. (2023). An AI dietitian for type 2 diabetes mellitus management based on large language and image recognition models: Preclinical concept validation study. *Journal of Medical Internet Research, 25*, e51300.

Thirunavukarasu, A. J., Hassan, R., Mahmood, S., Sanghera, R., Barzangi, K.,…& El Mukashfi, M. (2023). Trialling a large language model (ChatGPT) in general practice with the applied knowledge test: Observational study demonstrating opportunities and limitations in primary care. *JMIR Medical Education, 9*(1), e46599.

Tiase, V. L., & Cato, K. D. (2021). From artificial intelligence to augmented intelligence: Practical guidance for nurses. *OJIN: The Online Journal of Issues in Nursing, 26*(3), 4.

Tripathi, M., & Chandra, S. P. (2023). ChatGPT: A threat to the natural wisdom from artificial intelligence. *Neurology India, 71*(3), 416–417.

Wang, X., Sanders, H. M., Liu, Y., Seang, K., Tran, B. X., Atanasov, A. G., & Chung, K. C. (2023). ChatGPT: Promise and challenges for deployment in low- and middle-income countries. *The Lancet Regional Health–Western Pacific, 41*, 100905.

Author(s) Information

Rumyana Stoyanova
Medical University of Plovdiv
15a Vassil Aprilov Blvd., Plovdiv, Bulgaria
Contact e-mail: *Rumyana.Stoyanova@mu-plovdiv.bg*

Aleksandar Stoyanov
Technical University of Sofia
Student Complex, 11 Prof. Georgi Bradistilov Street, 1756 Sofia, Bulgaria

To cite this article:

Stoyanova, R., & Stoyanov, A. (2025). Evaluating the role of ChatGPT in health information provision: Capabilities, limitations, and ethical implications. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 39-46.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 47-57

ICGeHeS 2025: International Conference on General Health Sciences

Intermittent Fasting as a Halal Lifestyle: A Content Analysis

Muhamad Slamet Yahya
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Fahri Hidayat
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Mukhroji Mukhroji
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Atabik Atabik
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Asdlori Asdlori
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Fira Miftahul-Ulum
UIN Prof. K. H. Saifuddin Zuhri Purwokerto

Huzdaeni Rahmawati
UIN Sunan Kalijaga Yogyakarta

Abstract: The pandemic is gradually overcoming but this should not make us neglectful of health. There are several things that are a problem for people in Indonesia, namely obesity. This is not only due to the mindset of the people who think that fat is healthy, but also as a result of the pandemic which requires people to limit outdoor activities. This impaired mobility also reduces the daily rate of calorie burning. In addition, a lifestyle that is increasingly facilitated by the convenience of devices such as ordering food and drinks through applications makes calorie intake increase. This makes some people obese. In fact, in addition to reducing aesthetics, obesity can reduce the quality of life. Therefore, a breakthrough is needed to change the post-pandemic lifestyle, namely by implementing a halal lifestyle by fasting. In this study, the fasting in question is fasting Ramadan, fasting David, and fasting Monday-Thursday. This turns out to be in line with a diet method practiced by non-Muslims called *intermittent fasting.* The method used is qualitative with an *interpretive paradigm* on Youtube media. The analysis used is *reception analysis* —an analysis that examines the relationship between media content and the audience as an *active interpreter* who gives meaning to videos that combine the concept of *intermittent fasting* with fasting. The purpose of this research is as *exploratory research.* While the approach taken is integrative interconnective, namely combining the viewpoints of two branches of science, namely religion and science. The results obtained are that there is great potential that sunnah fasting can be applied as a halal lifestyle that has been studied for its benefits in terms of science. So, this does not make the branding of sunnah fasting a dogma but reaffirmation of sunnah fasting as a sensible halal lifestyle.

Keywords: Sunnah fasting, Halal lifestyle, Diet

Introduction

In carrying out daily activities, humans have a habit as a result of what they repeat continuously (Ritchie et al., 2003). This will then have an impact on human life. Habits that are repeated continuously can be said as a lifestyle. But for more details, some experts have the appropriate meaning of what a lifestyle is. From a psychological point of view, it explains that lifestyle is a form of compensation for imperfections in certain things, formed at the age of 3-5 years (Palacz-Poborczyk et al., 2025, p. 45), lifestyle can also be used to understand individual behavior, become the background of individual characteristics, and is determined by certain inferiority (Sunaryo, 2004, p. 109).

In addition to the above understanding, lifestyle can be used as a differentiator of individual characters from others (Bocu et al., 2024, p. 9). This is because each person has a different pattern of behavior. Lifestyle is also influenced by social organizational behavior related to consumption behavior (Mulyana, 2022, p. 72). Life is a series of choices. There is a saying that "You are what you eat". Therefore, we must be wise in choosing food and managing the right portion. This has a direct impact on the quality of life. Healthy lifestyle choices refer to intentional personal decisions to engage in behaviors that reduce or increase risk and disease. For example, choosing fasting as a lifestyle will have a good impact on health (Ernawati, 2022, p. 15).

Fasting is practiced by almost all religions in the world. Both Islam, Christianity, Catholicism, Buddhism, and Hinduism make fasting as one of worship (Francis et al., 2010, p. 146). In Islam, fasting is one of the pillars of Islam. It is undeniable that fasting has its own meaning for people who do it. Both in terms of spiritual and physical, fasting has a significant impact (Wahid, 2019, p. 2). As for the basis of the obligatory fast for every Muslim, namely:

اَيُّهَا الَّذِيْنَ اٰمَنُوْا لَيْكُمُ الصِّيَامُ ا لَى الَّذِيْنَ لِكُمْ لَعَلَّكُمْ

"O you who believe, fasting is prescribed for you as it was prescribed for those before you so that you may become pious." (Surah Al-Baqarah, n.d.)

The virtues of fasting were narrated by the Prophet Muhammad, as follows:

"From Abu Hurairah RA said: The Messenger of Allah, said: Allah said: Except fasting, the deeds are for Me and I will repay them. Because he has left his lust and his food because of Me. For people who fast get two happiness, namely happiness when breaking the fast and meeting their Lord." (HR Muslim) (Bukhari & Muslim, 2017, p. 497).

The wisdom of carrying out sunnah fasting is to train oneself from lust, teach simplicity, maintain health, train oneself to get used to istiqomah, and get the pleasure of being part of the Prophet's ummah. (Aidah, 2021, pp. 49–50). Meanwhile, in terms of health, fasting also has a good impact. Based on research, fasting can beat at least 25 diseases including bronchitis, headaches, constipation, hypertension, diabetes, obesity, cancer, etc. In addition, it is also mentioned that the crime rate decreases during the month of Ramadan. Therefore, it is interesting to study how fasting can be used as a lifestyle for people, especially after the pandemic (Azwar, 2005, pp. 58–59).

Based on the research of Kristin K. Hoddy, et al., explained that the Intermittent Fasting Approach can provide health benefits independent of weight loss. This is consistent with a meta-analysis showing decreased fasting insulin (9,115) although there was no difference in weight loss between the IF and CR regimens (9-10,86,115). It's possible that one IF regimen could outperform another, but this remains to be directly tested. Nonetheless, repeated samples across each type of IF regimen did show an insulin-sensitizing effect occurring soon after diet initiation. It is possible that this IF effect could be mediated through circadian biology as diurnal variation in glucose (116), energy expenditure (117,118), and substrate utilization (119) favoring eating in the morning and fasting at night (Hoddy et al., 2020, p. 534).

Based on research from Anisa Cherif, et al., explained that exercise has an impact on brain function, but information about the effects of a combination of exercise and IF (CR, RIF and IF) on cognitive function is scarce. Research has revealed that regular exercise improves certain types of learning, including executive function of cognition, learning, and memory span, and also stimulates neurogenesis. In addition, depending on the type of IF, cognitive function and physical performance may be enhanced or negatively impacted. Several studies have shown that long-term food restriction is associated with impairments in cognitive function, including poor performance on sustained attention tasks. However, other studies have shown that memory performance is significantly improved during fasting. In investigating the mechanism by which dietary restriction acts on cognitive function and to determine how this diet works (Cherif et al., 2016, p. 44).

Based on research from Santos and Mecido stated that IF may be a dietary method to help improve lipid profiles in healthy men and women, obesity and dyslipidemia, reduce total cholesterol, LDL, triglycerides and increase HDL. However, most studies analyzing the impact of IF on lipid profile and weight loss are observational and lack detailed information on diet. Randomized clinical trials with larger sample sizes are needed to evaluate the effect of IF especially in patients with dyslipidemia (Santos & Macedo, 2018, p. 20).

Method

This research is qualitative in nature, which is general in nature, changing, or developing according to field conditions. This method is also often referred to as naturalistic research because the research is carried out in settings or conditions natural ones. This method is positivistic and uses interpretive techniques. Researchers are tasked with interpreting the data found in the field (Setiawan, 2018, p. 17). The analysis used is reception analysis, an analysis that examines the relationship between media content and the audience as an active interpreter who gives meaning to videos that combine the concept of intermittent fasting with sunnah fasting (James, 2010, p. 59). The approach used in this research is an integrative-interconnective approach. The integrative-interconnective approach is an approach that seeks to respect each other's general knowledge and religion, being aware of each other's limitations in solving human problems. This will give birth to a collaboration, at least understanding each other's approach (approach) and method of thinking (process and procedure) between the two disciplines (Abdullah, 2006, p. 33). The primary data used is a video from the Diet Santuy YouTube channel entitled "How to Intermittent Fasting / OCD, Fasting Weight Loss Without Dieting" with a duration of 10 minutes 19 seconds. Data collection techniques using observation, documentation, and literature study. Observations were made by observing the object of research, namely in terms of content and interaction between viewers in the video commentary session. Documentation is carried out as a reinforcement to the data collected. Literature Study is a method of collecting data sourced from reference books, journals, papers, websites and readings that are related to research titles that can support problem solving obtained in research (Hartono, 2017, p. 16).

Results and Discussion

Correlation between Ramadan Fasting, Sunnah Monday-Thursday, Sunnah Fasting of David and Intermittent Fasting

The basis for the obligatory fasting of Ramadan has been mentioned in the previous chapter, namely al-Baqarah verse 183. Fasting Ramadan is one of the obligations of worship for every Muslim who is able to withstand hunger, thirst, and things that invalidate the fast from sunrise to sunset (Suwaidân, 2013, p. 174). The basis for the Sunnah fasting Monday-Thursday is the hadith narrated by the Prophet Muhammad, as follows:

.اَلاَعْمَالُ الاِثْنَيْنِ الخَمِيْسِ اُحِبُّ اَنْ لِيْ اَنَا ائِمٍ

"*Deeds are exposed (to Allah) on Mondays and Thursdays, so I like it if my deeds are exposed when I am fasting.*" (Narrated by Bukhari Muslim)

From the hadith above, it is found that there is wisdom for those who fast on Monday-Thursday so that our final deeds are good in front of Allah. While the basis for the stipulation of the Sunnah of David fasting, namely,

.اَحَبُّ الصِّيَامِ اِلَى اللهِ امُ اوُدَ انَ ا ا

"*The fast that Allah loves the most is the fast of the Prophet David. He fasts a day and does not fast for a day.*" (Narrated by Bukhari).

From the above hadith, it can be seen that David's fasting is the most beloved fasting practice by Allah SWT, (Abdillah, 2021, pp. 142–144). So what is the procedure for implementing these two sunnah fasts? First, the sunnah fasting Monday-Thursday is actually almost the same as the obligatory fast during the month of Ramadan (Fatahi et al., 2021, p. 3391). However, what distinguishes it is in the reading of the intention as follows,

.الصَّوْمَ الإِثْنَيْنِ\الخَمِيْسِ لِلَّهِ الَى

"*I intentionally fast Monday/Thursday because of Allah ta'ala."*

As for the intention of fasting David as the reading below,

.الصَّوْمَ اوُدَ لِلَّهِ الَى

"*Accidentally I fasted David kana Allah ta'ala*"

The two sunnah fasts above have the same procedure as obligatory fasting in general, namely refraining from everything that invalidates the fast from sunrise to sunset. It's just that both have different execution times (Nonaka, 2021, p. 437). Monday-Thursday sunnah fasting is carried out on Mondays and Thursdays outside of the fasting time of Ramadan. While the fast of David is carried out by fasting one day while the next day is not fasting (Hambali, 2020, pp. 283–285).

So, what about intermittent fasting? Intermittent fasting is a diet method using fasting (Diastiti, 2017, p. 9). Intermittent fasting is an eating pattern in which there is a cycle between periods of eating and fasting which is known to have the goal of losing weight and controlling several chronic diseases (Litaay et al., 2021, p. 97). Intermittent fasting has many benefits. There is even a Greek proverb which says that it is better to fast than taking medicine today (Correale, 2022, p. 90).

Based on research from Rafael and Mark on the effectiveness of intermittent fasting in health, aging, and disease, this has a good impact (Dalvi & Medithi, 2024, p. 241). Preclinical studies and clinical trials have shown that intermittent fasting has a broad spectrum of benefits for many health conditions, such as: obesity, diabetes mellitus, cardiovascular disease, cancer, and neurological disorders (Lassi & Bhutta, 2013, p. 652). In animals it is shown that intermittent fasting improves health throughout the life span, whereas clinical research mainly involves short-term interventions, over a period of several months (Mishra et al., 2024, p. 759). In addition, clinical studies have focused primarily on overweight young and middle-aged adults, and we cannot generalize to other age groups the benefits and safety of intermittent fasting that have been observed in these studies (Naaman et al., 2024, p. 8). Although it is not fully understood the specific mechanism, the beneficial effects of intermittent fasting involve metabolic switching and cellular stress resistance. However, some people are unable or unwilling to adhere to a regimen of intermittent fasting. By better understanding the processes linking intermittent fasting with broad health benefits, we may be able to develop targeted pharmacological therapies that mimic the effects of intermittent fasting without the need to substantially change feeding habits (Ahmed et al., 2018, p. 2706). Studies of the mechanisms of calorie restriction and intermittent fasting in animal models have led to the development and testing of pharmacological interventions that mimic the health and disease-modifying benefits of intermittent fasting. (de Cabo & Mattson, 2019, p. 2549)

Even in other studies intermittent fasting has an impact on several diseases. Patient demographic data, history of seizures, and seizure frequency. Six children attempted intermittent fasting and ranged in age from 2 to 7 years (Cars & Craig, 1990, p. 134). All families offer an agreed-upon intermittent fasting approach to try. The diagnoses included Lennox-Gastaut syndrome (two patients), Doose syndrome (two patients), and one child each with idiopathic generalized epilepsy and multifocal epilepsy (Fernández-Rodríguez et al., 2023, p. 11173). All patients except one were in a 4:1 ketogenic ratio and all were in KD for at least 4 months, which is sufficient time to determine whether KD has worked as the only dietary treatment for seizures. Interestingly, all children who had a positive response to the intermittent fasting regimen experienced atonic seizures as part of their semiology (Wilhelmi De Toledo et al., 2020, p. 152). Only two children (patients 2 and 6) did not increase seizures with KD on intermittent fasting. Other children experienced some degree of seizure improvement, but not to a degree of seizure freedom. (Hartman et al., 2013, p. 276)

Diet Santuy YouTube Content on Intermittent Fasting and Sunnah Fasting

According to Anton Septian—creator of the Diet Santuy Youtube channel—explains in his video that the meaning of intermittent fasting in language means intermittent fasting (Gueldich et al., 2019, p. 703). Meanwhile, in terms, intermittent fasting is an approach to regulating eating patterns by applying intermittent fasting and eating times (Karras et al., 2023, p. 264). There are three well-known approaches to intermittent fasting. First, the alternate day approach—that is, a diet method by fasting one day, one day after eating. In a religious context it is called the fast of David.

Figure 1. Fasting monday-thursday

Second, the 5:2 fasting approach—that is, within a week there are two days of fasting followed by five days of eating. In a religious context it is known as fasting Monday-Thursday. Although in intermittent fasting there is no need to fast for the two days mentioned.

Figure 2. David's fast

Third, the time-restricted feeding approach—that is, the fasting approach with limited eating hours (Cuccolo et al., 2022, p. 480). In a matter of one day there are hours to fast and there are hours to eat. Usually, the progression of this diet is to reduce eating hours gradually (Marventano et al., 2018, p. 103). For example, in the early stages of fasting for 12 hours, then the remaining 12 hours are for eating. Gradually the fasting time was increased, and the meal time was reduced (Sandoval et al., 2021, p. 740). For example, in the next stage it becomes 14 hours of fasting and 10 hours of eating. Some extreme people even eat only a small amount of time. People call it one meal a day because they only eat once a day. It is not recommended for the general public. This time-restricted feeding approach is similar to fasting during Ramadan—that is, a few hours for sahur and eating after fasting. The difference is in intermittent fasting it is permissible to drink water, while in Ramadan fasting it is not allowed to drink.

Figure 3. Ramadan fasting

The effectiveness of intermittent fasting with a restricted time feeding approach has been scientifically tested. In the first study, there were 20 obese people and had metabolic syndrome parameters. These 20 people fasted for 14 hours and the remaining 10 hours to eat. The result, after 12 weeks of fasting, the average study participants experienced a decrease in caloric intake by 8.6% and managed to lose weight as much as 3.3 kg. The second study fasted for 16 hours so the remaining 8 hours for eating. The participants were 40 people with 20 people fasting while the remaining 20 people eating as usual. After 12 weeks the people who were asked to fast had a calorie deficit of 341 calories and lost 2.6% of their weight. Then the next study used only 4 hours to eat, 6 hours, and the last group did their usual eating pattern. The study participants were 60 people grouped into 3 patterns as previously mentioned. The results after 8 weeks found that the group that ate only 4 hours a day experienced a calorie deficit of 528 calories, while the group that ate only 6 hours a day experienced a calorie deficit of 566 calories. And his weight managed to drop as much as 3.2%. So, from this research it can be concluded that this is effectively done. Because indirectly, by fasting food intake is reduced.

The advantage of intermittent fasting is that it is very easy to do. We don't need to count calories or other weird methods to lose weight (Jin et al., 2024). The drawback is that we must be ready to withstand hunger, affecting activities and life style, if the standard meal hours are carried out freely without having good nutritional literacy, this could be in vain or the nutritional intake eaten is not sufficient for the body's needs. Intermittent fasting does not recommend eating carelessly during meal times. If the hours of eating are short, it will be difficult for us to meet nutritional needs. If there is a tendency to eating disorders, it is not recommended to fast. This is because intermittent fasting applies skipping meals. Skipping meals to lose weight is considered dangerous for people with eating disorders (Nizami et al., 2022, p. 500).

There are several suggestions that can be applied in intermittent fasting. First, choose a reasonable eating window to start intermittent fasting. Second, stick to a good nutrition plan. Do not vent hunger by eating food that exceeds the body's needs. Third, intermittent fasting can be executed with a simple approach. For example, it's like a myth to stop eating after 18.00 or at night because it can make the body fat. Even though this is a hoax, it can be applied in the context of intermittent fasting. This has indirectly implemented intermittent fasting because they have fasted for 8 hours of fasting. At the time of eating can be added high-fiber foods to hold hunger longer. Lastly, please adjust it to the life style that has been applied (Diet Santuy, 2021)

Content Analysis and Viewers' Conception of the Diet Santuy towards Intermittent Fasting and Fasting

Detailed explanations about fasting and intermittent fasting managed to attract the hearts of the audience. This is proven by the video which has been watched 1824,012 times since it was uploaded on May 1, 2021. Not only that, the video also managed to get 5600 likes, and 691 people actively participated in providing feedback in the comment column.

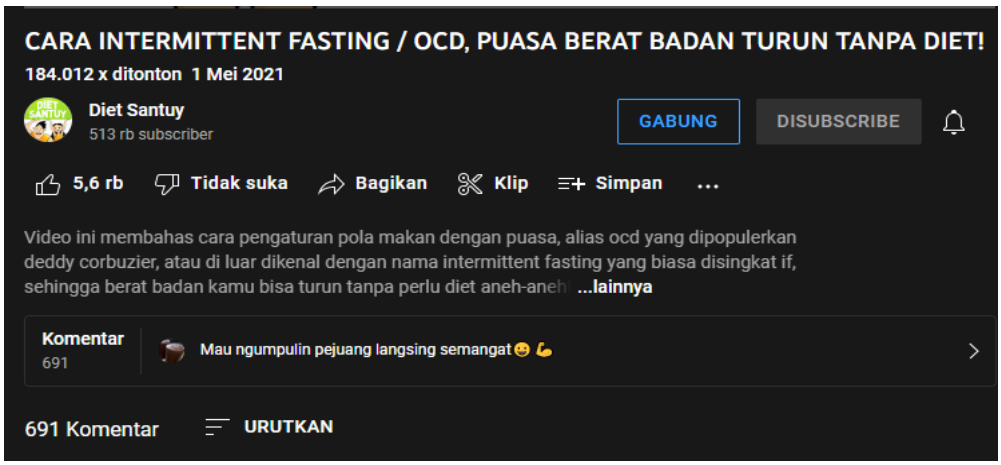

Figure 4. Proof of number of likes on content

From here we have seen a potential how the concept of intermittent fasting and fasting will educate the public at a wider stage. There are several positive things that make the Diet Santuy worthy of applause from the audience, including:

Presentation Display with Attractive Animation

The Diet Santuy gives a presentation screen-like appearance with dominant colors of green and white. This is also supported by animated characters whose visuals are eye-catching. In addition to its beauty, the images displayed represent the messages that are conveyed well. As stated by Roland Barthes—a philosopher from France—he explained that images have a trick effect. This is defined as the process of manipulating images to convey messages (Andhita, 2021, p. 55). In addition, in the context of education, visual media is one of the most widely used learning media because of the ease of conveying the meaning of the message content to the audience (Sartika et al., 2022, p. 72). So, the diet santuy has succeeded in displaying its educational content so that it is easy to understand and not boring.

Straightforward Delivery Accompanied by Relevant Examples

Anton Septian managed to explain intermittent fasting and fasting in a straightforward manner. His monologue did not feel monotonous because he invited the audience to react several times. For example, as in the 32nd second section as follows

Figure 5. The uniqueness of the content that makes the content interesting

This certainly brings the audience's interest up, because they feel involved in the discussion (Al-Tabany, 2017, p. 137). In addition, an interesting discussion of the diet santuy is also accompanied by examples that are relevant to the audience. For example, an explanation related to the myth that it is forbidden to eat at night. Bringing context into everyday life is certainly successful in making it easier for the audience to understand what is meant by the creator (Parera, 2004, p. 47)

Combining Explanations in Religious and Scientific Contexts

In the diet Santuy narrative about intermittent fasting, two approaches are used at once. The first is a religious approach and the second uses a scientific approach. Although the term intermittent fasting comes from non-Muslim circles, Anton Septian managed to find similarities in fasting. This is explained in three intermittent fasting approaches. In addition, education about research data is explained in a simple way to make it easier to understand. This is a reinforcement of the reason fasting is very well applied as a lifestyle (Tinsley & Horne, 2018, p. 49).

Presenting the Objective Side by Explaining the Advantages and Disadvantages

The explanation of intermittent fasting by presenting a religious and scientific context provides reinforcement for suggestions for the audience to do. This will lead to the decision to overdo it. In addition, it can encourage fanaticism and ridicule to others who choose not to do so. Therefore, it is very interesting in the video to explain the advantages and disadvantages of doing a fasting diet or intermittent fasting (Yehya et al., 2023, p. 453).

From the four reasons above, it can be understood how the diet Santuy deserves praise for its educational content. On the other hand, this can provide a new concept of intermittent fasting, which is a foreign term that is

very close to the religious context. This is evidenced by several comments from the audience that support the content. As mentioned by the owner of the Gading Aurora account,

"*If the intention is to fast, usually it can last until sunset, but if the intention is IF, the stomach is usually rumbling in broad daylight. In the end, until now, I prefer sunnah fasting (Monday-Thursday, ayyamul bidh, etc.). Anyway, it all depends on the intention.*"

This is also supported by comments from the owner of the Harely Lusiana account which states that

"*Once, Sis, I think it's better to watch your mealtime, because you can adjust your busyness with the incoming food. For those who fast intermittently or 2 days a week, it can be adjusted to the hours of commotion/laying down which are more than activities to rest the body, or it's not bad to save money when it comes to boarding children.*"

But on the other hand, this also creates a difference of opinion which states that intermittent fasting and fasting are two different things. As Chiro de Kocheng mentioned,

"*Fasting for me is essentially different when fasting is worship, the bonus is that it has a diet and detox effect, but in my religion, fasting is not allowed every day of the year except for fasting in Ramadan which can be full for a month, so if you want to diet, choose a relaxed diet, only this year I'm learning fasting is brought along with the diet, correcting the eating pattern. Because sometimes fasting just takes worship, iftar is out of control, it's lazy to eat at dawn. Now trying to follow a better pattern.*"

From this it can be understood that the content of the diet santuy has presented a new concept about fasting. From a religious point of view, fasting is a form of worship that has other benefits. This is reinforced by the support of scientific explanations for the reasons why intermittent fasting is good to do. With the reach of content that has been watched more than 1000 times, of course, this can be a potential to make fasting branding rise to the next level. Not only dogma about obligations or sunnah recommendations, but can be used as a new lifestyle. Especially in a post-pandemic situation, this can be the best solution to introduce fasting as a halal lifestyle (Heiat et al., 2021, p. 29).

Conclusion

Intermittent fasting literally means intermittent fasting. Meanwhile, based on the term, intermittent fasting is a diet method that uses fasting as a way to lose weight. The three approaches to intermittent fasting are similar to the fasting worship performed by Muslims in general. The first alternate day is similar to the concept of David's fasting. Secondly, 5:2 approaches are similar to the Monday-Thursday fasting approach. Thirdly, time restricted feeding methods are similar to the Ramadan fasting method. There are several things that distinguish Islamic fasting from intermittent fasting, namely the intention of the implementation, in the implementation of fasting it is not allowed to eat and drink; while intermittent fasting is allowed to drink as long as it is low in calories, fasting is done at a time determined by sharia; While intermittent fasting can be done at a time that has been planned by each individual as needed. In the diet santuy content about intermittent fasting, it was successful in introducing this diet method by bringing a religious context. This brings the image of fasting which has only been seen as one of the pillars of Islam into a sensible lifestyle. This is because the content of the diet santuy does not only present a religious context but is supported by a scientific context. So, this is able to lead the audience's conception to be more objective.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

*Thank you to LPDP for funding this research so that it can be presented directly on site.

Acknowledgements or Notes

*This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Abdillah, S. (2021). *Risalah puasa*. Guepedia.

Abdullah, M. A. (2006). *Islamic studies di perguruan tinggi: Pendekatan integratif-interkonektif.* Pustaka Pelajar.

Ahmed, A., Saeed, F., Arshad, M. U., Afzaal, M., Imran, A., Ali, S. W., Niaz, B., Ahmad, A., & Imran, M. (2018). Impact of intermittent fasting on human health: An extended review of metabolic cascades. *International Journal of Food Properties, 21*(1), 2700–2713.

Aidah, S. N. (2021). *Panduan praktis menjalankan puasa sunah.* Indonesia: Penerbit Kbm.

Al-Baqarah, S. (n.d.). *Qur'an kemenag*. Retrieved from https://quran.kemenag.go.id/surah/2/183

Al-Tabany, T. I. B. (2017). *Mendesain model pembelajaran inovatif, progresif, dan* konteksual. Indonesia: Prenada Media.

Andhita, P. R. (2021). *Komunikasi visual*. Zahira Media Publisher.

Azwar, B. (2005). *Manfaat puasa menurut ilmu kesehatan*. Kawan Pustaka.

Bocu, K., Boeri, L., Mahmutoglu, A. M., & Vogiatzi, P. (2024). Can lifestyle changes significantly improve male fertility: A narrative review? *Arab Journal of Urology,* 1–11.

Bukhari, I., & Muslim, I. (2017). *Hadits shahih Bukhari - Muslim (HC).* Indonesia: Elex Media Komputindo.

Cars, O., & Craig, W. A. (1990). Pharmacodynamics of Antibiotics-Consequences for Dosing. *Scandinavian Journal of Infectious Diseases, 22*(74), 1–284.

Cherif, A., Roelands, B., Meeusen, R., & Chamari, K. (2016). Effects of intermittent fasting, caloric restriction, and Ramadan intermittent fasting on cognitive performance at rest and during exercise in adults. *Sports Medicine, 46*(1), 35–47.

Correale, A. (2022). *Intermittent fasting: The complete step by step guide for men and women for easy weight loss with 16/8 method (workout routine and delicious healthy recipes included).* Indonesia: Anna Correale.

Cuccolo, K., Kramer, R., Petros, T., & Thoennes, M. (2022). Intermittent fasting implementation and association with eating disorder symptomatology. *Eating Disorders, 30*(5), 471–491.

Dalvi, M., & Medithi, S. (2024). Dietary factors in circadian rhythm modulation and their impact on metabolic diseases: A state of the science review. *Biological Rhythm Research, 55*(3–4), 233–259.

de Cabo, R., & Mattson, M. P. (2019). Effects of Intermittent Fasting on Health, Aging, and Disease. *New England Journal of Medicine, 381*(26), 2541–2551.

Diastiti, N. (2017). *Intermittent fasting: Turunkan berat badan dengan berpuasa.* Indonesia: Anak Hebat.

Diet Santuy. (2021). Cara intermittent fasting. Retrieved from https://www.youtube.com/watch?v=KPjIP1V3iks

Ernawati, D. (2022). *Kebiasaan Sehat—Perubahan gaya hidup sederhana untuk anda yang lebih sehat.* Indonesia: Lilin Novia.

Fatahi, S., Nazary-Vannani, A., Sohouli, M. H., Mokhtari, Z., Kord-Varkaneh, H., Moodi, V., Tan, S. C., Low, T. Y., Zanghelini, F., & Shidfar, F. (2021). The effect of fasting and energy restricting diets on markers of glucose and insulin controls: A systematic review and meta-analysis of randomized controlled trials. *Critical Reviews in Food Science and Nutrition, 61*(20), 3383–3394.

Fernández-Rodríguez, R., Martínez-Vizcaíno, V., Mesas, A. E., Notario-Pacheco, B., Medrano, M., & Heilbronn, L. K. (2023). Does intermittent fasting impact mental disorders? A systematic review with meta-analysis. *Critical Reviews in Food Science and Nutrition, 63*(32), 11169–11184.

Francis, L. J., Robbins, M., & Cargas, S. (2010). The parliament of the world's religions: Who goes and why? An empirical study of Barcelona 2004. *Journal of Beliefs & Values, 31*(2), 143–153.

Gueldich, H., Zghal, F., Borji, R., Chtourou, H., Sahli, S., & Rebai, H. (2019). The effects of Ramadan intermittent fasting on the underlying mechanisms of force production capacity during maximal isometric voluntary contraction. *Chronobiology International, 36*(5), 698–708.

Hambali, M. (2020). *Panduan Muslim kaffah sehari-hari dari kandungan hingga kematian.* Indonesia: Laksana.

Hartman, A. L., Rubenstein, J. E., & Kossoff, E. H. (2013). Intermittent fasting: A "new" historical strategy for controlling seizures? *Epilepsy Research, 104*(3), 275–279.

Hartono, J. (2017). *Metoda pengumpulan dan teknik analisis data*. Indonesia:Penerbit Andi.

Heiat, M., Hashemi-Aghdam, M.-R., Heiat, F., Rastegar Shariat Panahi, M., Aghamollaei, H., Moosazadeh Moghaddam, M., Sathyapalan, T., Ranjbar, R., & Sahebkar, A. (2021). Integrative role of traditional and modern technologies to combat covid-19. *Expert Review of Anti-Infective Therapy, 19*(1), 23–33.

Hoddy, K. K., Marlatt, K. L., Çetinkaya, H., & Ravussin, E. (2020). Intermittent fasting and metabolic health: from religious fast to time-restricted feeding. *Obesity, 28*(1), 29–37.

James, W. (2010). *Pragmatism: A New name for some old ways of thinking.* The Floating Press.

Jin, S., Chen, P., Yang, J., Li, D., Liu, X., Zhang, Y., Xia, Q., Li, Y., Chen, G., Li, Y., Tong, Y., Yu, W., Fan, X., & Lin, H. (2024). Phocaeicola vulgatus alleviates diet-induced metabolic dysfunction-associated steatotic liver disease progression by downregulating histone acetylation level via 3-HPAA. *Gut Microbes, 16*(1), 2309683.

Karras, S. N., Koufakis, T., Adamidou, L., Dimakopoulos, G., Karalazou, P., Thisiadou, K., Zebekakis, P., Makedou, K., & Kotsa, K. (2023). Different patterns of changes in free 25-hydroxyvitamin D concentrations during intermittent fasting among meat eaters and non-meat eaters and correlations with amino acid intake. *International Journal of Food Sciences and Nutrition, 74*(2), 257–267.

Lassi, Z. S., & Bhutta, Z. A. (2013). Risk factors and interventions related to maternal and pre-pregnancy obesity, pre-diabetes and diabetes for maternal, fetal and neonatal outcomes: A systematic review. *Expert Review of Obstetrics & Gynecology, 8*(6), 639–660.

Litaay, C., Paotiana, M., Elisanti, E., Fitriyani, D., Agus, P. P., Permadhi, I., Indira, A., Puspasari, G., Hidayat, M., Priyanti, E., & Darsono, L. (2021). *Kebutuhan gizi seimbang*. Zahir Publishing.

Marventano, S., Godos, J., Platania, A., Galvano, F., Mistretta, A., & Grosso, G. (2018). Mediterranean diet adherence in the Mediterranean healthy eating, aging and lifestyle (meal) study cohort. *International Journal of Food Sciences and Nutrition, 69*(1), 100–107.

Mishra, A., Sobha, D., Patel, D., & Suresh, P. S. (2024). Intermittent fasting in health and disease. *Archives of Physiology and Biochemistry, 130*(6), 755–767.

Mulyana, A. (2022). *Gaya hidup metroseksual: Perspektif komunikatif*. Indonesia:Bumi Aksara.

Naaman, R. K., Alashmali, S., Bakhsh, M. A., Muqaibil, A. A., Ghunaim, F. M., & Alattas, A. H. (2024). Association of omega-3 polyunsaturated fatty acids intake and cognitive function in middle-aged and older adults. *Nutritional Neuroscience*, *28*(6),649-658.

Nizami, H., Su, L., Jain, R., & Jain, R. (2022). Effects of chronically skipping meals on atrial fibrillation risk. *Future Cardiology, 18*(6), 497–506.

Nonaka, Y. (2021). Practising Sunnah for reward of heaven in the afterlife: The expansion of cadar wearing among urban Muslim women in Indonesia. *Indonesia and the Malay World, 49*(145), 429–447.

Palacz-Poborczyk, I., Chamberlain,K., Naughton, F., Baska, A., Luszczynska ,A., Quested, E., Hagger,M. S., Pagoto,S., Verboon,P., Robinson, S., & Kwasnicka, D. (2025). 'A healthy lifestyle is a journey': Exploring health perceptions and self-defined facilitators to health through photo-elicitation. *Psychology & Health, 40*(4), 652–680.

Parera, J. D. (2004). *Teori semantik.* Indonesia: Erlangga.

Ritchie, G., Still, K., Bekkedal, M., Bobb, A., & Arfsten, D. (2003). Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *Journal of Toxicology and Environmental Health, Part B*, *6*(4),357-451.

Sandoval, C., Santibañez, S., & Villagrán, F. (2021). Effectiveness of intermittent fasting to potentiate weight loss or muscle gains in humans younger than 60 years old: A systematic review. *International Journal of Food Sciences and Nutrition, 72*(6), 734–745.

Santos, H. O., & Macedo, R. C. O. (2018). Impact of intermittent fasting on the lipid profile: Assessment associated with diet and weight loss. *Clinical Nutrition ESPEN, 24*, 14–21.

Sartika, S. H., Subakti, H., Salamun, S., Chamidah, D., Firdian, F., Nirbita, B. N., Kuswandi, S., Arianti, I., Simarmata, J., & Mansyur, M. Z. (2022). *Teknologi dan media dalam pembelajaran.* Indonesia: Yayasan Kita Menulis.

Setiawan, A. A., Johan. (2018). *Metodologi penelitian kualitatif*. Jejak Publishing.

Sunaryo, S. (2004). *Psikologi.* EGC.

Suwaidân, T. M. (2013). *Rahasia puasa menurut 4 mazhab.* Indonesia:Maghfirah Pustaka.

Tinsley, G. M., & Horne, B. D. (2018). Intermittent fasting and cardiovascular disease: current evidence and unresolved questions. *Future Cardiology, 14*(1), 47–54.

Wahid, A. (2019). *Rahasia dan keutamaan puasa sunah.* Indonesia:Anak Hebat.

Wilhelmi De Toledo, F., Grundler, F., Sirtori, C. R., & Ruscica, M. (2020). Unravelling the health effects of fasting: A long road from obesity treatment to healthy life span increase and improved cognition. *Annals of Medicine, 52*(5), 147–161.

Yehya, Y. M., El-Said, Z. H., Adel, M., Othman, B. H., Mansour, A. A., & Gad, S. M. (2023). Intermittent fasting and probiotics in non-alcoholic fatty liver in rats: Interplay between FGF19 and FGF21. *Egyptian Journal of Basic and Applied Sciences, 10*(1), 447–459.

Author(s) Information

Muhamad Slamet Yahya
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia
Contact e-mail: *Msyahya0410@uinsaizu.ac.id*

Fahri Hidayat
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

Mukhroji Mukhroji
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

Atabik Atabik
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

Asdlori Asdlori
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

Fira Miftahul Ulum
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

Huzdaeni Rahmawati
UIN Prof KH Saifuddin Zuhri Purwokerto
JL A Yani No 40A Purwokerto, Indonesia

To cite this article:

Yahya, M. S., Hidayat, F., Mukhroji, M., Atabik, A., Asdlori, A., Miftahul-Ulum, F., & Rahmawati, H. (2025). Intermittent fasting as a halal lifestyle: A content analysis. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 47-57.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 58-64

ICGeHeS 2025: International Conference on General Health Sciences

New Approaches to the Identification of Epigenetic Modifications in Chronic Lymphoid Leukemia

Ayse Dalyan
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Chronic Lymphoid Leukemia (CLL), one of the most common hematologic malignancies in adults in Western countries, is a disease in which epigenetic modifications play an important role in its pathogenesis. Fundamental epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNAs and are critical for normal cellular processes, including cellular growth, development, and differentiation. The identification and understanding of these modifications allows us to analyze the biology of the disease in more detail. Thanks to the technological approaches developed in recent years, it is possible to examine epigenetic changes in CLL in a more sensitive and detailed way. These methods provide a deeper understanding of the molecular mechanisms of the disease. In addition to being an important tool in the identification of epigenetic modifications in CLL, these new approaches also contribute to the development of diagnostic, prognostic, and therapeutic strategies. These developments will allow CLL to be managed more effectively.

Keywords: CLL, Epigenetic modifications, Histone modifications

Introduction

Chronic Lymphoid Leukemia (CLL), one of the most common hematologic malignancies in adults in Western countries, is a heterogeneous disease that develops with the interaction of genetic and epigenetic factors (Coll-Mulet & Gil, 2009; Chiorazzi et al., 2021). The disease has an incidence of about 4.2 cases per 100,000 people per year, with a median age at diagnosis of 70-72 years and a male prevalence of approximately 2:1 (Pérez-Carretero et al., 2021).

Epigenetic modifications include processes that alter gene expression without affecting the DNA sequence, such as DNA methylation, histone modifications and non-coding RNA (ncRNA) (Taylor et al., 2013; Prabhakaran et al., 2021). These modifications underlie many biological processes such as cell differentiation, adhesion and replication (Wu et al., 2023). Abnormal progression of epigenetic mechanisms can play an important role in the development of many diseases such as cancer. Abnormal epigenetic mechanisms may play important roles at various stages of tumor development, triggering the formation or spread of cancer (Dai et al., 2024).

In mammalian cells, DNA methylation occurs at specific sites, often called CpG islands, where CpG dinucleotides are concentrated (Tari et al., 2018). This methylation occurs mostly in gene promoter regions and often leads to gene expression arrest by repressing promoter activity (Tari et al., 2018; Hornschuh et al.,2021). Studies suggest that DNA methylation may have an important impact on the biology of CLL. (Landau & Wu, 2013; Bagacean et al., 2017).

DNA is tightly packaged with histone proteins to form the chromatin structure. In this process, DNA, approximately 147 base pairs long, is wrapped 1.7 turns around a core of 8 histone proteins (Hornschuh et

al.,2021). Histone can undergo different post-translational modifications such as acetylation, methylation, ubiquitination and phosphorylation and play important roles in the regulation of chromatin structure and expression of genes (Nie et al., 2024; Sherif et al., 2025). Abnormalities in histone modifications can lead to inhibition of tumor suppressor genes or activation of oncogenes in cancer development (Sherif et al., 2025).

Non-coding RNAs (ncRNAs) are RNA molecules that do not code for proteins but can regulate gene expression in multiple ways (Kumar et al., 2020). They are divided into two main groups: small non-coding RNAs (sncRNA) and long non-coding RNAs (lncRNA) (Sarropoulou & Fernández, 2023). MicroRNAs, small non-coding RNAs, regulate gene expression after transcription. Dysregulation of miRNAs contributes to the development of CLL (Mansouri et al., 2018).

The rapid advancement of epigenetic research has increased interest in new technologies that will enable a more detailed understanding of epigenetic changes in health and disease states (Li, 2021; Sherif et al., 2025). In this process, various methods have been developed that have the capacity to examine chromatin structures in many dimensions, from analyses focusing on specific gene regions to sequencing covering the entire genome. These advances are supported by many strategies such as advanced imaging systems, high-throughput sequencing technologies, and integration of bioinformatics tools (Li, 2021).

Emerging technologies in the field of epigenetics offer significant opportunities to better understand epigenetic modifications in CLL and translate this knowledge into clinical practice. In this study, we will focus on new approaches used to identify epigenetic modifications in CLL and their contribution to disease biology.

New Approaches to the Identification of Epigenetic Modifications

Next Generation Sequencing (NGS) Technology

In the late 1970s, DNA sequencing techniques emerged with the use of Sanger's sequencing by Edward Sanger and chemical fragmentation by Maxam-Gilbert. In 2005, Roche introduced the faster “454” technology. This technology is called “Next Generation Sequencing (NGS)” or “High Throughput Sequencing” (Mandlik et al., 2024). NGS enables the acquisition of large amounts of genetic information in a short time with high efficiency (Yin et al., 2021; Mandlik et al., 2024). The introduction of advanced NGS platforms such as Pacific Biosciences, Illumina, and Oxford Nanopore has revolutionized genomic research by enabling the parallel sequencing of large numbers (millions to billions) of DNA templates (Yin et al., 2021; Satam et al., 2023). These technologies offer new discovery opportunities to understand gene expression, genetic diversity, microbial diversity, and epigenetic modifications (Satam et al., 2023).

In recent years, with the use of next-generation sequencing technologies, significant progress has been made in understanding CLL at the molecular level. The genetic and epigenetic diversity of the disease has been revealed in detail, especially thanks to large-scale studies involving more than a thousand patients (Nagler et al., 2023). The development of NGS technologies has helped us to understand the biological complexity of CLL more comprehensively by allowing detailed examination of genomic, epigenomic and transcriptomic profiles (Nagler et al., 2023; Oder et al., 2023). NGS-based methods frequently used in epigenetic analyses are given below.

Whole Genome Bisulfite Sequencing (WGBS): WGBS is a gold standard method that can detect and quantitatively analyze DNA methylation at genome-wide base resolution (Luo et al., 2023; Yu et al., 2024). In 2009, the first human genome-wide single base resolution DNA methylation map was created using this method (Lister et al., 2009). However, WGBS has been used in basic and clinical studies to investigate the relationship between DNA methylation loci and human phenotypes (Zhou et al., 2019). Despite reductions in sequencing costs, the high cost of implementation for large genomes is an important limitation of the method (Ortega-Recalde et al., 2021; Luo et al., 2023).

Reduced Representation Bisulfite Sequencing (RRBS): RRBS, an efficient and high-throughput method for analyzing genome-wide methylation patterns, combines restriction enzymes and bisulfite sequencing to enrich regions with high CpG density (Bell &Wan, 2020; Nkongolo & Michael 2024). This technique was developed in 2005 by Meisner and colleagues to sequence a smaller representative sample of the entire genome, due to the high cost of sequencing for whole genome methylation analyses (Meissner et al., 2005). RRBS is a method for DNA methylation detection that is convenient in terms of low cost and time saving (Sun & Zhu, 2021).

Chromatin Immunoprecipitation sequencing (ChIP-seq): ChIP-seq is a method for analyzing genome-wide DNA-protein interactions in mammalian genomes, including histone modifications and transcription factors (Islam, 2021; Coskunpınar &Yıldırım, 2024). The integration of transcription factors and histone modifications provides a significant advantage for this technique in analyzing interactions between different genomic data (Coskunpınar &Yıldırım, 2024).

Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq): ATAC-seq is an efficient method for mapping chromatin accessibility in the genome (Buenrostro et al., 2015). ATAC-seq has been rapidly adopted because it is more efficient in terms of cost, time and the amount of samples required compared to previous tests (Smith & Sheffield, 2020).

RNA sequencing (RNA-seq): RNA-seq is a method used to determine which genes are expressed at what level in the cells of an organism at any stage of its life cycle. Transcriptome; all of the transcript types found in cells under certain conditions are called (Darcan & Turkyılmaz, 2018). RNA-seq with NGS technology has many advantages over previous methods and has led to radical changes in our understanding of the transcriptome. This method allows for a more detailed and quantitative examination of gene expression levels, alternative splicing events and allele-specific expression (Kukurba & Montgomery, 2015).

Single-Cell Sequencing Technology

In 2009, the first single-cell RNA sequencing method was developed (Tang et al., 2009). Since then, researchers have introduced new methods to improve the accuracy, resolution and throughput of sequencing. Today, sequencing at the single cell level provides in-depth insights into cellular diversity and function, contributing to the development of new strategies for the diagnosis and treatment of diseases (Xie et al., 2024). Single cell technologies enable detailed analysis of various biological modalities such as chromatin accessibility (scATAC-seq) and gene expression (scRNA-seq) at the resolution of individual cells. These approaches provide significant advantages over bulk data analysis, particularly in determining clonal structure and cell type distribution in the tumor microenvironment (Tsagiopoulou & Gut, 2024). With the increasing number of studies on single cell technologies in CLL, the genomic, epigenetic and transcriptional landscape in CLL has begun to be elucidated at the single cell level (Gohil & Wu, 2019).

CRISPR Technology

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology has been optimized for high-throughput screening, manipulation, and precise epigenome editing of epigenetic regulators. This technology allows researchers to alter specific epigenetic markers by intervening in targeted genomic regions. This method allows functional analyses of epigenetic modifications and also offers potential for therapeutic purposes by reactivating tumor suppressor genes (Sherif et al., 2025).

CRISPR associated protein 9 (Cas9) system functions as an RNA-dependent nuclease and is known by its abbreviation as CRISPR/Cas9 (Dalai & Sar, 2021). In 2013, this technology was used for the first time for genome editing in mammalian cells and spread rapidly. Thanks to its ability to regulate gene expression, ranging from genome sequence changes to epigenetic and transcriptional modifications, this technology has found significant application (Hernández-Sánchez, 2022).

Artificial Intelligence Technology

Artificial intelligence, in its most basic form, is a technology that enables computer systems to perform tasks that require human intelligence. These tasks include complex processes such as speech recognition, natural language understanding, and decision making. Deep learning (DL) and Machine Learning (ML) are subcategories of artificial intelligence (Ozcelik et al., 2024).

Artificial intelligence enables significant advances in precision medicine related to cancer. AI technologies can save time by automating the initial analysis of images, as well as extracting features by examining stained tumor slices or radiological data. These systems can distinguish cancerous and non-cancerous cells with high accuracy and can be effective in defining tumor morphology, dimensions, subtype, and level of spread (Yu et al., 2024).

Machine learning is a branch of artificial intelligence that makes predictions about future events by analyzing large data sets (Rauschert et al., 2020). Deep Learning is a subset of machine learning that uses neural networks to process large data sets to gain insights (Ozcelik et al., 2024). ML applications have significantly contributed to the investigation of many questions regarding the basic biological functions of epigenetic elements, their contributions to gene regulation processes, and the potential benefits of the epigenome in cancer diagnosis and treatment (Arslan et al., 2021).

Examples of Literature Studies Conducted with Epigenetic Methods in CLL

Epigenetic modifications such as promoter hypermethylation can drive cancer by causing tumor suppressor genes to lose their function. Pan et al. (2021) reported DNA methylation driver cells in CLL by regulating DNA methylation with CRISPR. They suppressed the expression of three candidate drivers, DUSP22, RPRM and SASH1, in the HG3 cell line and changed promoter methylation with the dCas9 system (Pan et al., 2021).

Pastore and colleagues conducted a comprehensive study to delve deeper into the epigenetic aspect of cancer evolution. To this end, they used bulk reduced representation bisulfite sequencing (RRBS) analysis, a chromatin immunoprecipitation sequencing (ChIP-seq) method of histone post-translational modifications and gene expression, and performed joint DNA methylation and transcriptome single-cell analysis. The study found that coordination between different layers of the CLL epigenome was significantly reduced. This ongoing epigenetic diversification has reportedly led to a mixture of cells with different epigenetic profiles, providing new insight into the epigenetic dimension of cancer evolution (Pastore et al., 2019). By applying multiplex single-cell reduced representation bisulfite sequencing (MscRRBS) to B cells from CLL patients and healthy donors, Gaiti and colleagues showed that epigenetic information enables mapping of CLL lineage history and prediction of its evolution after therapy (Gaiti et al., 2019).

Knisbacher and colleagues conducted a study integrating genomic, transcriptomic and epigenomic data to create a map of CLL. Combining existing and new data, the largest dataset to date, including WES/WGS, RNA-seq and DNA methylation analyses, was generated from samples of 1095 CLL and 54 MBL patients. These comprehensive data have contributed to a better definition of the molecular subtypes and biological features of CLL.This study provides new insights into CLL oncogenesis and prognosis in general (Knisbacher et al., 2022).

Mallm and colleagues performed a comprehensive analysis of chromatin modifications, comparing aberrant epigenetic mechanisms in primary CLL cells with NBCs. This research predicts new molecular connections to targets of CLL therapies and provides an important resource for further studies on the epigenetic contribution to the disease (Mallm et al., 2019)

Grimm et al performed a study of 79 German patients to evaluate the clinical utility of an epigenetic marker set for CLL patient stratification. The study used pyrosequencing and support vector machine learning tool following bisulfite treatment. Researchers have confirmed the prognostic value of epigenetic classification in CLL and reported that it is useful for patient stratification in the clinic (Grimm et al., 2022).

Conclusion

Identification and understanding of epigenetic modifications in CLL allows for a deeper understanding of the biological mechanisms of the disease. The use of new technologies allows these modifications to be studied in more detail, contributing to significant advances in the diagnosis, prognosis and treatment of CLL In the future, further advancement of epigenetic research will pave the way for the development of more targeted, personalized treatment strategies for CLL, enabling more effective management of CLL.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* The authors received no financial support for this study.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

References

Arslan, E., Schulz, J., & Rai, K. (2021). Machine learning in epigenomics: Insights into cancer biology and medicine. *Biochimica et Biophysica Acta Reviews on Cancer*, *1876*(2), 188588.

Bagacean, C., Tempescul, A., Le Dantec, C., Bordron, A., Mohr, A., Saad, H., Olivier, V., Zdrenghea, M., Cristea, V., Cartron, P. F., Douet-Guilbert, N., Berthou, C., & Renaudineau, Y. (2017). Alterations in DNA methylation/demethylation intermediates predict clinical outcome in chronic lymphocytic leukemia. *Oncotarget*, *8*(39), 65699–65716.

Buenrostro, J. D., Wu, B., Chang, H. Y., & Greenleaf, W. J. (2015). ATAC-seq: A method for assaying chromatin accessibility genome-wide. *Current Protocols in Molecular Biology, 109,* 2129.

Chiorazzi, N., Chen, S. S., & Rai, K. R. (2021). Chronic lymphocytic leukemia. *Cold Spring Harbor Perspectives in Medicine*, *11*(2), a035220.

Coll-Mulet, L., & Gil, J. (2009). Genetic alterations in chronic lymphocytic leukaemia. *Clinical & Translational Oncology: Official Publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico*, *11*(4), 194–198.

Dai, W., Qiao, X., Fang, Y., Guo, R., Bai, P., Liu, S., Li, T., Jiang, Y., Wei, S., Na, Z., Xiao, X., & Li, D. (2024). Epigenetics-targeted drugs: Current paradigms and future challenges. *Signal Transduction and Targeted Therapy*, *9*(1), 332.

Darcan, C., & Turkyılmaz, O., (2018). Overview of next generation sequencing technology. *Bilecik Seyh Edebali Universitesi Fen Bilimleri Dergisi*, *5*(1), 41-49

Gaiti, F., Chaligne, R., Gu, H., Brand, R. M., Kothen-Hill, S., Schulman, R. C., Grigorev, K., Risso, D., Kim, K. T., Pastore, A., Huang, K. Y., Alonso, A., Sheridan, C., Omans, N. D., Biederstedt, E., Clement, K., Wang, L., Felsenfeld, J. A., Bhavsar, E. B., Aryee, M. J., …& Landau, D. A. (2019). Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia. *Nature*, *569*(7757), 576–580.

Gohil, S. H., & Wu, C. J. (2019). Dissecting CLL through high-dimensional single-cell technologies. *Blood*, *133*(13), 1446–1456.

Grimm, C., Herling, C. D., Komnidi, A., Hussong, M., Kreuzer, K. A., Hallek, M., & Schweiger, M. R. (2022). Evaluation of a prognostic epigenetic classification system in chronic lymphocytic leukemia patients. *Biomarker Insights*, *17*, 11772719211067972.

Hernández-Sánchez, M. (2022). CRISPR/Cas9 in Chronic Lymphocytic Leukemia. *Encyclopedia*, *2*(2), 928-936

Hornschuh, M., Wirthgen, E., Wolfien, M., Singh, K. P., Wolkenhauer, O., & Däbritz, J. (2021). The role of epigenetic modifications for the pathogenesis of Crohn's disease. *Clinical Epigenetics*, *13*(1), 108.

Islam, R. (2021). *Epigenetic dysregulation in lymphoid leukemias.* (Unpublished doctoral dissertation). University of British Columbia.

Knisbacher, B. A., Lin, Z., Hahn, C. K., Nadeu, F., Duran-Ferrer, M., Stevenson, K. E., Tausch, E., Delgado, J., Barbera-Mourelle, A., Taylor-Weiner, A., Bousquets-Muñoz, P., Diaz-Navarro, A., Dunford, A., Anand, S., Kretzmer, H., Gutierrez-Abril, J., López-Tamargo, S., Fernandes, S. M., Sun, C., Sivina, M., Getz, G. (2022). Molecular map of chronic lymphocytic leukemia and its impact on outcome. *Nature Genetics*, *54*(11), 1664–1674.

Kukurba, K. R., & Montgomery, S. B. (2015). RNA sequencing and analysis. *Cold Spring Harbor Protocols*, *2015*(11), 951–969.

Kumar, S., Gonzalez, E. A., Rameshwar, P., & Etchegaray, J. P. (2020). Non-coding RNAs as mediators of epigenetic changes in malignancies. *Cancers*, *12*(12), 3657.

Landau, D. A., & Wu, C. J. (2013). Chronic lymphocytic leukemia: molecular heterogeneity revealed by high-throughput genomics. *Genome Medicine*, *5*(5), 47.

Li, Y. (2021). Modern epigenetics methods in biological research. *Methods (San Diego, Calif.)*, *187*, 104–113.

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., Nery, J. R., Lee, L., Ye, Z., Ngo, Q. M., Edsall, L., Antosiewicz-Bourget, J., Stewart, R., Ruotti, V., Millar, A. H., Thomson, J. A., Ren, B., & Ecker, J. R. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, *462*(7271), 315–322.

Luo, X., Wang, Y., Zou, Q., & Xu, L. (2023). Recall DNA methylation levels at low coverage sites using a CNN model in WGBS. *PLoS Computational Biology*, *19*(6), e1011205.

Mallm, J. P., Iskar, M., Ishaque, N., Klett, L. C., Kugler, S. J., Muino, J. M., Teif, V. B., Poos, A. M., Großmann, S., Erdel, F., Tavernari, D., Koser, S. D., Schumacher, S., Brors, B., Konig, R., Remondini, D., Vingron, M., Stilgenbauer, S., Lichter, P., Zapatka, M., …& Rippe, K. (2019). Linking aberrant chromatin features in chronic lymphocytic leukemia to transcription factor networks. *Molecular Systems Biology*, *15*(5), e8339.

Mandlik, J. S., Patil, A. S., & Singh, S. (2024). Next-generation sequencing (NGS): Platforms and applications. *Journal of Pharmacy & Bioallied Sciences*, *16*(Suppl 1), S41–S45.

Mansouri, L., Wierzbinska, J. A., Plass, C., & Rosenquist, R. (2018). Epigenetic deregulation in chronic lymphocytic leukemia: Clinical and biological impact. *Seminars in Cancer Biology*, *51*, 1–11.

Meissner, A., Gnirke, A., Bell, G. W., Ramsahoye, B., Lander, E. S., & Jaenisch, R. (2005). Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. *Nucleic Acids Research*, *33*(18), 5868-5877.

Nagler, A., & Wu, C. J. (2023). The end of the beginning: application of single-cell sequencing to chronic lymphocytic leukemia. *Blood*, *141*(4), 369–379.

Nie, H., Kong, X., Song, X., Guo, X., Li, Z., Fan, C., Zhai, B., Yang, X., & Wang, Y. (2024). Roles of histone post-translational modifications in meiosis†. *Biology of Reproduction*, *110*(4), 648–659.

Nkongolo, K., & Michael, P. (2024). Reduced representation bisulfite sequencing (RRBS) analysis reveals variation in distribution and levels of DNA methylation in white birch (*Betula papyrifera*) exposed to nickel. *Genome*, *67*(10), 351–367.

Oder, B., Chatzidimitriou, A., Langerak, A. W., Rosenquist, R., & Osterholm, C. (2023). Recent revelations and future directions using single-cell technologies in chronic lymphocytic leukemia. *Frontiers in Oncology*, *13*, 1143811.

Ortega-Recalde, O., Peat, J. R., Bond, D. M., & Hore, T. A. (2021). Estimating global methylation and erasure using low-coverage whole-genome bisulfite sequencing (WGBS). *Methods in Molecular Biology (Clifton, N.J.)*, *2272*, 29–44.

Ozcelik, F., Dundar, M. S., Yildirim, A. B., Henehan, G., Vicente, O., Sánchez-Alcázar, J. A., Gokce, N., Yildirim, D. T., Bingol, N. N., Karanfilska, D. P., Bertelli, M., Pojskic, L., Ercan, M., Kellermayer, M., Sahin, I. O., Greiner-Tollersrud, O. K., Tan, B., Martin, D., Marks, R., Prakash, S., …& Dundar, M. (2024). The impact and future of artificial intelligence in medical genetics and molecular medicine: an ongoing revolution. *Functional & Integrative Genomics*, *24*(4), 138.

Pan, H., Renaud, L., Chaligne, R., Bloehdorn, J., Tausch, E., Mertens, D., Fink, A. M., Fischer, K., Zhang, C., Betel, D., Gnirke, A., Imielinski, M., Moreaux, J., Hallek, M., Meissner, A., Stilgenbauer, S., Wu, C. J., Elemento, O., & Landau, D. A. (2021). Discovery of candidate DNA methylation cancer driver genes. *Cancer Discovery*, *11*(9), 2266–2281.

Pastore, A., Gaiti, F., Lu, S. X., Brand, R. M., Kulm, S., Chaligne, R., Gu, H., Huang, K. Y., Stamenova, E. K., Béguelin, W., Jiang, Y., Schulman, R. C., Kim, K. T., Alonso, A., Allan, J. N., Furman, R. R., Gnirke, A., Wu, C. J., Melnick, A. M., Meissner, A., … & Landau, D. A. (2019). Corrupted coordination of epigenetic modifications leads to diverging chromatin states and transcriptional heterogeneity in CLL. *Nature Communications*, *10*(1), 1874.

Pérez-Carretero, C., González-Gascón-Y-Marín, I., Rodríguez-Vicente, A. E., Quijada-Álamo, M., Hernández-Rivas, J. Á., Hernández-Sánchez, M., & Hernández-Rivas, J. M. (2021). The evolving landscape of chronic lymphocytic leukemia on diagnosis, prognosis and treatment. *Diagnostics*, *11*(5), 853.

Prabhakaran, R., Thamarai, R., Sivasamy, S., Dhandayuthapani, S., Batra, J., Kamaraj, C., Karthik, K., Shah, M. A., & Mallik, S. (2024). Epigenetic frontiers: miRNAs, long non-coding RNAs and nanomaterials are pioneering to cancer therapy. *Epigenetics & Chromatin*, *17*(1), 31.

Rauschert, S., Raubenheimer, K., Melton, P. E., & Huang, R. C. (2020). Machine learning and clinical epigenetics: a review of challenges for diagnosis and classification. *Clinical Epigenetics*, *12*(1), 51.

Sar, P., & Dalai, S. (2021). CRISPR/Cas9 in epigenetics studies of health and disease. *Progress in Molecular Biology and Translational Science*, *181*, 309–343.

Sarropoulou, E., & Fernández, I. (2023). Epigenetic regulation of gene expression by noncoding RNAs. In F. Piferrer & H. P. Wang (Eds.), *Epigenetics in aquaculture* (pp. 65–93). Wiley.

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R. P., Banday, S., Mishra, A. K., Das, G., & Malonia, S. K. (2023). Next-generation sequencing technology: Current trends and advancements. *Biology*, *12*(7), 997.

Sherif, Z. A., Ogunwobi, O. O., & Ressom, H. W. (2025). Mechanisms and technologies in cancer epigenetics. *Frontiers in Oncology*, *14*, 1513654.

Smith, J. P., & Sheffield, N. C. (2020). Analytical approaches for ATAC-seq data analysis. Current *Protocols in Human Genetics, 106*(1), e101.

Sun, R., & Zhu, P. (2021). Advances in measuring DNA methylation. *Blood science (Baltimore, Md.)*, *4*(1), 8–15.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., Siddiqui, A., Lao, K., & Surani, M. A. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. *Nature methods*, *6*(5), 377–382.

Tari, K., Shamsi, Z., Reza Ghafari, H., Atashi, A., Shahjahani, M., & Abroun, S. (2018). The role of the genetic abnormalities, epigenetic and microRNA in the prognosis of chronic lymphocytic leukemia. *Experimental Oncology*, *40*(4), 261–267.

Taylor, K. H., Briley, A., Wang, Z., Cheng, J., Shi, H., & Caldwell, C. W. (2013). Aberrant epigenetic gene regulation in lymphoid malignancies. *Seminars in Hematology*, *50*(1), 38–47.

Tsagiopoulou, M., & Gut, I. G. (2024). Machine learning and multi-omics data in chronic lymphocytic leukemia: the future of precision medicine?. *Frontiers in Genetics*, *14*, 1304661.

Wan, M., & Bell, D. A. (2020). Analysis of genome-wide methylation using reduced representation bisulfite sequencing (RRBS) technology. In *Epigenetics methods* (pp. 141–156). London, UK: Elsevier.

Wu, Y. L., Lin, Z. J., Li, C. C., Lin, X., Shan, S. K., Guo, B., Zheng, M. H., Li, F., Yuan, L. Q., & Li, Z. H. (2023). Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. *Signal Transduction and Targeted Therapy*, *8*(1), 98.

Xie, D., An, B., Yang, M., Wang, L., Guo, M., Luo, H., Huang, S., & Sun, F. (2024). Application and research progress of single cell sequencing technology in leukemia. *Frontiers in oncology*, *14*, 1389468.

Yıldırım, H., & Coskunpınar, E. M. (2024). Epigenetik ve epigenomik. In H. Hosni Sobhi Alsaadoni & M. Beker (Eds.), *Saglık & bilim 2024: Tıbbi biyoloji ve genetik*-I (pp. 19-38). Turkiye:Efe Akademi Publishing.

Yin, Y., Butler, C., & Zhang, Q. (2021). Challenges in the application of NGS in the clinical laboratory. *Human Immunology*, *82*(11), 812–819.

Yu, X., Zhao, H., Wang, R., Chen, Y., Ouyang, X., Li, W., Sun, Y., & Peng, A. (2024). Cancer epigenetics: from laboratory studies and clinical trials to precision medicine. *Cell Death Discovery*, *10*(1), 28.

Zhou, L., Ng, H. K., Drautz-Moses, D. I., Schuster, S. C., Beck, S., Kim, C., Chambers, J. C., & Loh, M. (2019). Systematic evaluation of library preparation methods and sequencing platforms for high-throughput whole genome bisulfite sequencing. *Scientific Reports*, *9*(1), 10383.

Author(s) Information

Ayse Dalyan
University of Gaziantep Department of Biology, 27310 Gaziantep, Türkiye
Contact e-mail: *agurlekdalyan@gmail.com*

Mehmet Ozaslan
University of Gaziantep Department of Biology, 27310 Gaziantep, Türkiye

To cite this article:

Dalyan, A., & Ozaslan, M. (2025). New approaches to the identification of epigenetic modifications in chronic lymphoid leukemia. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 58-64.

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 65-72

ICGeHeS 2025: International Conference on General Health Sciences

Building Eco-Awareness in Preschool Children

Rizki Feroza Maruddani
Universitas Pendidikan Indonesia

Raoda Tul Jannah Maruddani
Institut Agama Islam Muhammad Azim Jambi

Mamat Ruhimat
Universitas Pendidikan Indonesia

Abstract: Environmental education plays a vital role in fostering ecological awareness and sustainable behavior from an early age. In Indonesia, preschool education offers a strategic platform to cultivate eco-consciousness among young learners. However, its integration faces significant challenges, including limited resources, inadequate teacher training, and the absence of structured curricula. This study explores how Indonesian preschool teachers implement environmental education by examining their strategies, obstacles, and key success factors. Employing a qualitative case study approach, data were gathered through online semi-structured interviews with 15 preschool teachers from various regions, along with curriculum and lesson plan analyses. Thematic analysis revealed that while teachers highly value environmental education, their efforts are often hindered by insufficient training, lack of materials, and rigid curricular frameworks. Despite these barriers, many teachers adopt innovative approaches such as project-based learning, outdoor exploration, and collaborative activities to engage students. Key enablers of effective environmental education include teacher motivation, strong school leadership, and parental involvement. This study highlights the urgent need for enhanced teacher preparation, better resource provision, and more structured curricular support. Addressing these areas is essential for fostering environmental stewardship in young Indonesian children. The findings offer practical insights for educators, policymakers, and curriculum developers committed to advancing early childhood environmental education.

Keywords: Eco-Awareness, Environment, Environmental education

Introduction

Environmental education in early childhood is widely recognized as a crucial foundation for developing ecological awareness, responsible behavior, and sustainable mindsets from a young age. Early childhood, particularly the preschool years, is a formative period where values, attitudes, and habits toward the environment begin to take shape (Lamanauskas, 2023; Lithoxoidou et al., 2017). Educating children about nature and sustainability during this stage not only enhances their understanding of the natural world but also nurtures empathy, respect, and a sense of responsibility for the environment (Ernst et al., 2023; Sihvonen et al., 2024; Zhai et al., 2025;Ernst et al., 2023; Sihvonen et al., 2024; Zhai et al., 2025). Early exposure to these principles lays the foundation for children to grow into environmentally conscious individuals, equipped to make informed decisions and contribute positively to the sustainability of the planet.

Teachers play a central role in integrating environmental education into preschool curricula. Equipping educators with the knowledge and tools to teach environmental topics is essential for fostering young learners' understanding of sustainability and the natural world (Roussou et al., 2025). Effective strategies include hands-on activities,

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

storytelling, play, and child-centered pedagogical approaches. Early exposure to nature-based learning experiences, such as outdoor play, gardening, and recycling activities, has been shown to positively influence children's environmental attitudes and behaviors (Luo & Li, 2024). These activities not only teach children about ecological concepts but also cultivate empathy, curiosity, and a sense of responsibility toward the natural world.

Scaffolding, a method of providing structured guidance, enables young learners to grasp complex ecological concepts in a step-by-step manner (Zurek & Torquati, 2014). For example, using simple recycling tasks or nature-themed stories allows children to connect abstract environmental ideas to their everyday experiences. Additionally, interdisciplinary approaches, such as integrating environmental themes into science, math, and language activities, enhance cognitive and emotional development. The readiness and attitudes of teachers significantly impact the success of environmental education initiatives. Studies reveal that educators with higher levels of environmental literacy are more likely to adopt innovative and engaging teaching methods (Turkoglu, 2019). Professional development programs that focus on sustainability and environmental awareness equip teachers with the skills and confidence to implement effective eco-education strategies (Mliless et al., 2024). Moreover, fostering a collaborative learning environment where teachers and children explore environmental topics together promotes a shared sense of purpose and engagement (Kangas et al., 2017).

Sustainability can also be embedded into early childhood curricula through global perspectives and localized approaches. Across various countries, educators adopt unique strategies to address environmental challenges, offering valuable models for adaptation. Community and parental involvement further amplify the impact of environmental education. Encouraging families to participate in sustainable practices and supporting cmmunity-based initiatives creates a holistic learning ecosystem that reinforces lessons taught in the classroom. The benefits of early environmental literacy extend beyond ecological understanding. Research demonstrates that environmental education in early childhood enhances problem-solving skills, creativity, and social-emotional development (Carter, 2016) . Children exposed to these concepts are more likely to adopt environmentally friendly behaviors, such as conserving water and reducing waste, from a young age (Fortino et al., 2014). Furthermore, these foundational experiences lay the groundwork for developing environmentally conscious and responsible adults. In conclusion, building eco-awareness in preschool children through thoughtful teaching approaches is essential for fostering a generation committed to environmental sustainability. By combining effective pedagogical strategies, teacher training, and community collaboration, early childhood education can serve as a powerful platform for promoting environmental stewardship and shaping a sustainable future.

Environmental education (EE) is essential in fostering eco-consciousness and sustainable practices from an early age. In Indonesia, preschool education (ECE) provides a critical opportunity for young children to develop these values. However, the integration of environmental education into preschool curricula remains challenging due to factors like limited resources, lack of specialized teacher training, and inadequate environmental curriculum structures. Despite these barriers, the role of preschool teachers is central to promoting eco-awareness among young learners. Environmental education in early childhood plays a critical role in developing eco-awareness and sustainability attitudes that persist throughout life. Teachers in preschool settings are key facilitators of this early engagement, influencing young learners' attitudes toward nature, conservation, and environmental responsibility.

Research indicates that early experiences with nature can significantly shape children's attitudes toward the environment, promoting lifelong environmental stewardship. Various pedagogical approaches have been identified to effectively promote environmental awareness among young children. These include transformative and participatory methods, collaborative learning, and hands-on experiences. Professional development for educators, parental involvement, and community engagement are also essential components in successfully implementing environmental education in early childhood settings (Mliless et al., 2024).

Recent studies show that early childhood environmental education (ECEE) is vital for promoting eco-friendly behaviors and developing environmental citizenship (Poje et al., 2024; Rahmania, 2024). Teachers' approaches to this subject vary, but successful methodologies include hands-on learning, outdoor exploration, and thematic integration of environmental issues into daily activities (Davis, 2019). For instance, ECEE programs that incorporate local ecological systems and address climate change help instill a deeper sense of responsibility in children (Bennett & Daly, 2018). Furthermore, eco-conscious curricula often employ storytelling, games, and nature walks to connect children to their surroundings, strengthening their environmental knowledge and fostering empathy towards living creatures (Acar & Coskun, 2021).

Teachers' attitudes toward sustainability also shape the effectiveness of eco-awareness initiatives. Studies highlight that professional development and teacher training in environmental education enhance educators' confidence in integrating eco-conscious topics into the classroom (McNaughton, 2021). However, challenges remain, such as

time constraints and a lack of resources, which can limit the scope of environmental topics covered (Ellis & Hunt, 2022). Overall, building eco-awareness at the preschool level is not only an educational strategy but a proactive means of cultivating a generation of environmentally responsible individuals. As teachers implement and adapt various teaching methods, they provide crucial insights into how early childhood education can act as a foundational stage for environmental stewardship**.**

While environmental education is recognized as crucial for fostering eco-awareness, its effective integration into preschool education in Indonesia faces various challenges. Little is known about how preschool teachers implement environmental education in the classroom, the obstacles they encounter, or the factors that influence the success of these initiatives. This study aims to:

1. Investigate the strategies employed by preschool teachers to teach environmental education.
2. Identify the challenges teachers face when integrating environmental education into preschool settings.
3. Explore the factors that influence the effectiveness of environmental education in preschool classrooms.

This research provides valuable insights into how environmental education can be more effectively integrated into preschool education in Indonesia. It also offers guidance for policymakers, educators, and curriculum developers in improving teacher training and resource allocation for environmental education.

Method

Research Design

This study employs a qualitative case study approach to gain comprehensive insights into the experiences of preschool teachers in Indonesia. Utilizing online semi-structured interviews alongside document analysis, the research captures the personal perspectives of teachers as well as the contextual framework provided by educational materials. To ensure a diverse representation of teaching practices and experiences, 15 preschool teachers from different regions of Indonesia were selected based on their expertise in teaching preschool-aged children and their active participation in environmental education initiatives.

Data Collection Methods

To comprehensively gather insights and contextual understanding, this study utilized two primary data collection methods:

1) Online Semi-Structured Interviews: Interviews were conducted with preschool teachers through platforms such as Zoom and Google Meet. These interviews aimed to explore the teachers' approaches to environmental education, the challenges they encounter, and the strategies they implement to engage children in eco-friendly practices.

2) Document Analysis: Relevant curriculum materials, lesson plans, and school reports on environmental education were examined to understand how environmental education is structured within preschool settings and how teachers incorporate it into their teaching practices.

Data Analysis Procedures

The data obtained from interviews and document analysis were processed through thematic analysis to identify significant patterns and overarching themes. This analytical approach entailed several systematic stages, including familiarization with the data, generating initial codes, searching for themes, reviewing and refining these themes, and finalizing the thematic framework. The analysis specifically focused on categorizing key themes, such as effective teaching strategies, challenges faced by preschool educators, and influential factors contributing to the implementation of environmental education. By employing this iterative method, the study ensured a comprehensive and contextual interpretation of the findings, offering valuable insights into the practices and perspectives of preschool teachers in integrating environmental education into their teaching methodologies.

Ethical Considerations

This study adhered to ethical guidelines to ensure the rights and welfare of all participants were protected. Informed consent was obtained prior to their involvement, with participants fully briefed on the study's purpose, procedures, and their right to withdraw at any time without consequence. Confidentiality was rigorously maintained by anonymizing participants' identities and securely storing data to prevent unauthorized access. All responses were utilized solely for research purposes, ensuring transparency and adherence to ethical principles throughout the research process.

Results and Discussion

Teaching Strategies Used by Preschool Teachers

Preschool teachers utilize various strategies to integrate environmental education into their classrooms, emphasizing hands-on and experiential learning to spark curiosity, exploration, and active participation. They often introduce environmental concepts by focusing on the child's immediate surroundings, such as their home, school, and local community. For instance, children are encouraged to observe and explore their environment through activities like nature walks, observing plants and animals in their school or nearby areas. By anchoring lessons in familiar contexts, teachers help children build a sense of connection and responsibility toward their surroundings. Preschool children especially enjoy learning through direct, real-life experiences. Activities such as observing tangible objects in the school environment—like firsthand encounters with a drum (bedug) or a mosque dome are particularly engaging. Contextual, thematic, and project-based approaches, alongside role-playing activities, are among the most favored learning processes for young children. By directly interacting with their environment, children better comprehend the concepts being taught.

Figure 1. Preschool children actively participating in a planting activity

Early childhood education curricula are typically tailored to developmental stages, ensuring themes and sub-themes are age-appropriate and relevant. Teachers create safe and stimulating learning environments where children feel comfortable to explore and actively participate. These environments are organized to make learning spaces accessible, fostering independence and curiosity in children. Group activities, such as cleaning the classroom or schoolyard, are frequently implemented to instill environmentally conscious behaviors. Actions like proper waste disposal are reinforced through daily reminders, while eco-friendly habits, such as conserving energy and water, are practiced and modeled collectively. Teachers also introduce gardening and plant care to help children understand the importance of nurturing the natural world.

Storytelling with environmental themes is another effective method for teaching complex concepts like sustainability and conservation in a way that is engaging and age-appropriate. Interactive tools, such as recycling stations and eco-themed crafts, enable children to actively participate in environmentally friendly practices, turning abstract concepts into tangible experiences. Teachers often integrate local cultural values, such as *gotong royong* (mutual cooperation), to promote collective responsibility for environmental care.

To evaluate the effectiveness of these activities, teachers commonly use observations, checklists, and anecdotal records. Key indicators of success include children's enthusiasm during activities, their discipline, independence (such as throwing trash away without prompting), and the outcomes of their school environment-related projects. These assessment practices are designed to support and reflect each child's developmental progress comprehensively.

Through these thoughtfully designed learning experiences, preschool teachers nurture meaningful habits that resonate with children's developmental need for active and concrete learning. By embedding environmental themes into daily activities and lessons, educators lay a solid foundation for lifelong environmental awareness and responsibility in children.

Challenges Faced by Preschool Teachers

Preschool teachers encounter a multitude of challenges when attempting to integrate environmental education into their teaching practices. One of the most significant barriers is the difficulty in engaging young learners with environmental themes. Many preschoolers tend to prefer unstructured play and can exhibit limited interest in guided activities that involve abstract environmental concepts. This often makes it challenging for teachers to maintain their attention and enthusiasm for lessons related to sustainability, requiring the use of highly creative and interactive methods.

Another common issue is the limited availability of resources. Teachers frequently report a lack of access to relevant and up-to-date teaching materials, such as visual aids, interactive tools, or digital content, which can make it difficult to effectively deliver environmental education. As a result, educators often have to rely on their own creativity to develop materials, which can be both time-consuming and exhausting. Compounding this issue is the absence of a standardized environmental curriculum, leading to inconsistencies in how environmental education is approached and implemented across different schools and regions.

Behavioral challenges among children also present a significant hurdle. For example, teachers have noted that young learners may struggle to connect with abstract concepts, such as climate change or conservation, particularly when presented through stories or analogies. Instead, children often respond better to concrete examples and hands-on experiences, which require additional preparation and resources from teachers. The lack of specialized training and professional development opportunities further exacerbates these issues. Many teachers feel unequipped to introduce complex environmental topics in an age-appropriate manner due to insufficient training or access to best practices. This gap in knowledge and skills can impact the quality of environmental education provided to young children. Additionally, preschool teachers often face challenges in addressing diverse student needs, particularly when teaching children with special needs. Ensuring inclusivity while achieving comparable developmental progress for all children requires tailored approaches and additional support, which may not always be available. Institutional and administrative barriers also play a role. Limited support from school leadership or a lack of funding for environmental programs can leave teachers feeling isolated in their efforts to implement sustainable education. Differences in perspectives among educators, administrators, and parents further complicates the process, making it difficult to build a cohesive and well-supported environmental education program.

Lastly, broader environmental and societal issues can impact teaching efforts. For example, teachers in regions prone to natural disasters, such as flooding, often find these events disrupting school activities, while also underscoring the urgency and relevance of environmental education. Yet, without adequate resources or guidelines, connecting these real-world issues to learning objectives remains a challenge. Despite these obstacles, many preschool teachers display remarkable resilience, creativity, and dedication to fostering environmental awareness in their students. Addressing these challenges through systemic support—such as expanded training, improved access to resources, and clearer curriculum guidelines—would greatly enhance the effectiveness of environmental education in early childhood settings

Factors Influencing Effectiveness

The effectiveness of environmental education in preschool settings is shaped by a variety of interconnected factors. Teacher motivation plays a pivotal role, as educators who are passionate and informed about environmental issues tend to exhibit higher levels of engagement and creativity in their teaching methods. Their enthusiasm directly influences children's interest and understanding, creating a dynamic and interactive learning environment.

Institutional support is another critical factor. Schools with supportive leadership that prioritize environmental education by providing adequate resources, training opportunities, and encouragement enable teachers to integrate environmental themes more effectively into their curriculum. Access to appropriate teaching materials and administrative backing significantly reduces barriers to implementation.

Parental involvement further amplifies the effectiveness of environmental education. When parents actively participate by reinforcing environmental concepts at home—through practices like waste segregation, energy conservation, or gardening—children develop a stronger understanding and are more likely to adopt eco-friendly habits. Additionally, community engagement can greatly enhance educational efforts. Partnerships with local organizations, environmental groups, or community leaders provide access to additional resources, real-world experiences, and collaborative opportunities that enrich the learning process. For example, community-driven programs such as tree-planting events or clean-up drives can serve as practical learning extensions for children.

Lastly, policy and societal awareness also influence success. Educational policies that emphasize environmental sustainability and societal attitudes towards eco-conscious practices can create a more cohesive framework for both schools and families to promote environmental education. When schools operate in communities with higher levels of environmental awareness, they often receive greater support and collaboration in implementing sustainable programs. These factors underline the importance of a holistic approach, requiring collaboration among teachers, schools, families, and communities to establish a cohesive and supportive framework for environmental education. By addressing these elements collectively, preschool programs can more effectively nurture the next generation of environmentally conscious individuals.

Insights from Document Analysis

A review of school documents reveals a mixed and varied approach to environmental education across different preschool settings. While certain schools have made an effort to include environmental topics in their curricula, there is a significant lack of standardized guidelines for teaching these subjects. This inconsistency in approach results in uneven outcomes, as teachers are left to interpret and implement environmental education based on available resources. Unfortunately, in many cases, the materials provided are either outdated or overly simplistic, which limits their capacity to address the more complex and pressing issues of environmental sustainability. Teachers have voiced a clear need for updated, engaging, and comprehensive teaching materials that are tailored to the developmental needs of preschool children.

Despite these challenges, research highlights the critical benefits of Early Childhood Environmental Education (ECEE). Programs focusing on environmental education at an early age not only enhance children's cognitive skills, such as science literacy and problem-solving abilities, but also contribute to their social and emotional development. For instance, children exposed to environmental concepts often exhibit improved language acquisition, better environmental cognition, and the adoption of pro-environmental attitudes. These educational experiences lay a strong foundation for lifelong learning and sustainable practices, preparing young learners to become environmentally conscious citizens. Furthermore, ECEE has shown potential in nurturing empathy and collaboration among children, as they engage in activities like group recycling efforts or caring for plants. These interactions promote teamwork and foster a sense of responsibility for their surroundings, which can extend beyond the classroom into their daily lives.

Conclusion

This study identifies the significant challenges faced by preschool teachers in Indonesia when integrating environmental education, including a lack of training and resources. Despite these obstacles, many teachers show remarkable creativity, using innovative strategies to engage children in eco-friendly activities, supported by their motivation and community involvement. The findings emphasize the crucial role of teachers in fostering environmental awareness from an early age and the importance of systemic support to enhance their teaching impact.

In conclusion, building eco-awareness in preschool children through intentional and well-planned teaching approaches is vital to creating a generation that values and actively contributes to environmental sustainability. By applying effective teaching strategies and strengthening collaboration between teachers, parents, and communities, young learners can develop a strong foundation of environmental awareness.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Enhance Teacher Training Programs

Develop and implement professional development initiatives tailored to equip teachers with the necessary knowledge, skills, and confidence to effectively deliver environmental education. Such programs should include practical workshops, ongoing support, and access to best practices for teaching sustainability concepts to young learners.

2. Develop Resource-Supported Curricula

Design and provide comprehensive, age-appropriate curriculum materials and teaching frameworks that seamlessly integrate environmental topics into preschool activities. These resources should be adaptable and accessible, enabling teachers to overcome potential barriers such as resource scarcity or lack of institutional support.

3. Strengthen Collaborative Efforts

Encourage and facilitate partnerships between educational institutions, local communities, and environmental organizations. Collaborative initiatives can provide additional resources, share expertise, and create opportunities for experiential learning, such as community clean-ups or nature-based activities.

4. Expand Research Scope

Conduct further studies involving larger and more diverse participant groups, both within and beyond Indonesia, to gain broader insights into effective environmental education practices. Comparative analyses across different cultural and educational contexts can offer valuable perspectives for globally informed strategies.

5. Explore Parental Involvement

Examine the role of parents in reinforcing environmental education outside the classroom. Identifying effective methods for parental engagement, such as home-based activities or workshops, can amplify the overall impact of these educational efforts on children.

6. Evaluate Program Impacts

Systematically assess the long-term effects of environmental education initiatives on children's attitudes, behaviors, and understanding of sustainability. Measuring these outcomes can help refine educational strategies and ensure their effectiveness in cultivating environmentally conscious individuals.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest

Funding

* This research was financially supported by Indonesia Endowment Fund for Education (LPDP Indonesia), which provided substantial resources to ensure the completion of this study.

Acknowledgements or Notes

* This article was presented as an oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

*We extend our deepest gratitude to the Department of Geography Education at Universitas Pendidikan Indonesia for their invaluable guidance and support throughout the course of this research.

References

Carter, D. (2016). A nature-based social-emotional approach to supporting young children's holistic development in classrooms with and without walls: the social-emotional and environmental education development (seed) framework. *The International Journal of Early Childhood Environmental*

Education, *4*(1), 10–25.
Ernst, J., Underwood, C., Early, T. N.-I. J., undefined. (2023). of the Story: A Community of Practice Approach for Supporting Early Childhood Educators' Capacity for Fostering Empathy in Young Children through Nature-Based. *Eric*, *11*(1), 34.
Fortino, C., Gerretson, H., Button, L., & Masters, V. (2014). "Growing Up WILD": Teaching environmental education in early childhood. *International Journal of Early Childhood Environmental Education*, *2*(1), 156–171.
Homburg, A., & Stolberg, A. (2006). Explaining pro-environmental behavior with a cognitive theory of stress. *Journal of Environmental Psychology*, *26*(1), 1–14.
Kangas, M., Siklander, P., Randolph, J., & Ruokamo, H. (2017). Teachers' engagement and students' satisfaction with a playful learning environment. *Teaching and Teacher Education*, *63*, 274–284.
Lamanauskas, V. (2023). The ımportance of environmental education at an early age. *Journal of Baltic Science Education*, *22*(4), 564–567.
Lithoxoidou, L. S., Georgopoulos, A. D., Th Dimitriou, A., & Ch Xenitidou, S. (2017). "Trees have a soul too!" Developing Empathy and Environmental Values in Early Childhood. *The International Journal of Early Childhood Environmental Education*, *5*(1), 68–88.
Luo, H., & Li, W. C. (2024). Are They Ready for Sustainability? A Study of the Environmental Attitudes of Early Childhood In-Service Teachers. *Early Childhood Education Journal*.
Mliless, M., Larouz, M., & Azzouzi, L. (2024). *Environmental awareness as part of early childhood education for sustainability.* - *In* M. Mliless, M. Larouz, D. L. Forte, H. F. Halawachy, H. Handoko, & C. D. S. Vande-Guma (Eds.), *Environmental awareness in preschool children's drawings: A global perspective* (pp. 1–25). Cham: Springer Nature Switzerland.
Poje, M., Marinić, I., Stanisavljević, A., & Rechner Dika, I. (2024). Environmental education on sustainable principles in kindergartens—a foundation or an option? *Sustainability* , *16*(7), 1–15.
Rahmania, T. (2024). Exploring school environmental psychology in children and adolescents: The influence of environmental and psychosocial factors on sustainable behavior in Indonesia. *Heliyon*, *10*(18), e37881.
Roussou, A. M., Argyrakou, C. C., & Milakis, E. D. (2025). Integrating STEAM and theatrical methods in early childhood environmental education: A framework for holistic learning. *International Journal of Geography, Geology and Environment*, *7*(2), 19-42.
Sihvonen, P., Lappalainen, R., Herranen, J., & Aksela, M. (2024). Promoting Sustainability Together with Parents in Early Childhood Education. *Education Sciences*, *14*(5), 541.
Turkoglu, B. (2019). Opinions of preschool teachers and pre-service teachers on environmental education and environmental awareness for sustainable development in the preschool period. *Sustainability, 11*(18), 4925.
Zhai, J., Simone Miranda, B., Justin, D., Shanghao, W., & and Yan, X. (2025). Early childhood immersion in nature: Chinese kindergarten educators' perspectives on nature play. *Environmental Education Research*, *31*(2), 460–479.
Zurek, A., & Torquati, J. (2014). *Scaffolding as a Tool for Environmental Education in Early Childhood*. *2*(1), 27–57.

Author(s) Information

Rizki Feroza Maruddani
Universitas Pendidikan Indonesia
Bandung, West Java, Indonesia
Contact e-mail: *rizkiferoza@upi.edu*

Raoda Tul Jannah Maruddani
Institut Agama Islam Muhammad Azim
Jambi, Jambi, Indonesia

Mamat Ruhimat
Universitas Pendidikan Indonesia
Bandung, West Java, Indonesia

To cite this article:

Maruddani, R.F., Maruddani, R.T.J., & Ruhimat, M. (2025). Building eco-awareness in preschool children. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 17,* 65-72.