Volume 12 | 2026 Release

Journal of Asian Medical Students' Association

JAMSA
Journal of Asian Medical Students' Association

www.jamsa.amsa-international.org

Volume 12
August 2026
ISSN: 2226-3403

Journal of Asian Medical Students' Association

Volume 12 | 2026 Release

www.jamsa.amsa-international.org

Volume 12
August 2026
ISSN: 2226-3403

Editorial Page

The Journal of Asian Medical Students' Association (JAMSA) (ISSN: 2226- 3403) is an international, online open-access, peer-reviewed, student-led biomedical research journal of the Asian Medical Students' Association (AMSA) International. Established in 2011, JAMSA published its first issue in the year 2012. It is currently indexed in Ulrichsweb, Google Scholar, Index Copernicus and Gale Cengage Learning.

Attribution

- Cover photo by Vitaly Gariev on Unsplash
- Aurora Illustration by Mayliana Widhaningtyas and Paisan Mungkornsilanon on Vecteezy
- This book is set in Noto Sans for body text and Roboto Flex for display typography. Noto Sans was developed by The Noto Project Authors, and Roboto Flex was developed by Font Bureau, both for Google. Both fonts are licensed under the SIL Open Font License, Version 1.1.

Journal of Asian Medical Students' Association (JAMSA)
Number 100, Section 1, Jingmao Road, Beitun District, Taichung City 406040, Taiwan R.O.C.
j-amsa@amsa-international.org
ISSN: 2226-3403

Table of Contents

About JAMSA

Journal of Asian Medical Students' Association (JAMSA)

ISSN: 2226-3403

The Journal of Asian Medical Students' Association (JAMSA) is a peer-reviewed, international, and open-access biomedical journal led by students under the Asian Medical Students' Association-International (AMSA International). Published biannually, JAMSA is listed in the ICMJE and is a member of CrossRef. It is also indexed in reputable databases, including: Ulrichsweb, Google Scholar, ROAD (Directory of Open Access Scholarly Resources), Gale Cengage, BASE (Bielefeld Academic Search Engine), Genamics Journal Seek. JAMSA's vision is to promote student-led research throughout Asia, the Asia-Pacific region, and beyond.

Fostering Creativity in Medical Research

Medical student research is gaining global recognition, and AMSA International is committed to fostering this trend through its vision of Knowledge, Action, and Friendship. JAMSA encourages all forms of research and creative works from medical students, providing a platform for young, emerging researchers in the Asia-Pacific and other regions.

Scope of the Journal

The main goal of JAMSA is to document research activities across the medical student community. We encourage submissions in the form of: Original Research Articles, Review Articles, Case Reports, Feature Articles, Letters to the Editor. If you are interested in submitting a paper, please refer to the Author Guidelines and Submission Guidelines found on the Submission section of our website.

Who Can Submit?

JAMSA welcomes submissions from medical students worldwide, not limited to AMSA International members. We accept articles related to: All disciplines of medicine, Public health, Healthcare management. Any research with an impact on health is eligible for submission. However, the editorial board reserves the right to decline any article deemed unsuitable for publication. Our priority is to minimize the processing time for submissions, and our online submission and article processing system has been designed with efficiency in mind.

Get in Touch

For any questions or inquiries, please feel free to contact us on j-amsa@amsa-international.org

Foreword

Stephen Dario Syofyan

Chief Editor
of JAMSA 2025/2026

Dear Esteemed Readers and Future Contributors,

It is with great pride and honor that I present this Call for Papers for the upcoming issue of the Journal of Asian Medical Students' Association (JAMSA), focusing on the profound and intricate field of Neurology. As Chief Editor of JAMSA, I have had the privilege of witnessing the remarkable dedication, intellectual curiosity, and collaborative spirit of medical students across the Asia-Pacific region and beyond.

This issue's theme, centered on Neurology, reflects one of the most pressing and rapidly evolving frontiers in modern healthcare. With subthemes encompassing neurodegenerative diseases, clinical neurosciences, neurosurgery, and cognitive health, this Call for Papers underscores the complex and multifaceted ways in which neurological conditions shape overall wellbeing. These topics are not only timely but demand innovative, interdisciplinary approaches to ensure sustainable and equitable healthcare solutions.

The manuscripts we aim to compile in this journal will represent the collective efforts of some of the brightest young minds in medicine. We invite explorations of critical issues arising from neurological challenges, from diagnostic advancements to patient rehabilitation, while also proposing practical and forward-thinking solutions. This future body of work will stand as a testament to the commitment of future healthcare professionals to address emerging global health challenges with both scientific rigor and social responsibility.

The burden of neurological diseases continues to redefine the landscape of public health. Increasing aging populations, systemic barriers, and complex lifestyle factors contribute to a growing global incidence of neurological disorders. In this context, the research we seek to highlight emphasizes the urgent need for integrated strategies that bridge clinical neurology, public health, and policy-making.

I would like to extend my sincere anticipation and appreciation to all prospective authors, peer reviewers, and the editorial committee whose dedication and hard work will make this publication possible. Your future contributions will not only enrich academic discourse but also pave the way for meaningful impact in communities and clinical settings worldwide.

To all aspiring contributors of JAMSA, I encourage you to take full advantage of this platform to share your scientific discoveries, to collaborate, and to build lasting connections. May this Call for Papers inspire you to become proactive leaders and innovators in shaping a healthier, more resilient future in neurological care.

With my best wishes for a successful research and writing endeavor.

Viva AMSA!

Age-Dependent Effects of Chronic Unpredictable Stress on Male Reproductive Organs in Rats

Beheshteh Abouhamzeh[1], Kianoosh Gholami[2], Behnoush Sabayan[3], Mohammad Reza Ghasemzadeh[4], Parvin Zareian[5], Esmaeil Samizadeh[6]

[1]Department of Anatomical Sciences, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[2]Anesthesiology Resident, Department of Anesthesiology, AJA University of Medical Science, Tehran, Iran

[3]Ph.D. Candidate of Clinical Psychology, Department of Clinical Psychology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[4]Assistant Professor of Psychiatry, Department of Psychiatry, AJA University of Medical Sciences, Tehran, Iran

[5]Department of Physiology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran

[6]Pathology department of Imam Reza hospital, AJA University of Medical Sciences, Tehran, Iran

Background: Stress occurs in response to external harmful factors and can have detrimental effects on organ functions.

Objective: This study investigated the age-dependent effects of chronic unpredictable stress (CUS) on serum testosterone levels, testicular promyelocytic leukemia zinc finger (PLZF) expression, and histopathological changes in young and old male rats.

Methods: Thirty-two male Wistar rats were randomly assigned into four groups (young control, young stress, old control, and old stress; 8 rats in each group). Rats in the stress groups were subjected to one unpredictable stressor daily for eight consecutive weeks. Serum testosterone was measured using the Enzyme-Linked Immunosorbent Assay (ELISA), PLZF-positive spermatogonial cells were evaluated by immunohistochemistry (IHC), and testicular tissue was assessed using hematoxylin and eosin (H&E) staining. Data were analyzed using two-way ANOVA followed by Bonferroni post hoc testing.

Results: CUS significantly reduced serum testosterone levels in both stress groups in comparison to control groups ($p < 0.001$). Also, the proportion of PLZF-positive spermatogonial cells significantly decreased in the stress groups ($p < 0.001$). Histological results demonstrated reduced spermatogonia population in both stress groups while sloughing of seminiferous tubules was observed only in the old stress group.

Conclusion: CUS impairs endocrine and histological markers of the male reproductive system, with different responses based on age. These findings suggest that CUS may contribute to male infertility through changes in testosterone production, spermatogonial maintenance, and testicular histology.

Keywords: Chronic Unpredictable Stress, Testicular Tissue, Testosterone, Rats

Category: Original Research

Date Received: 02 June 2026

Date Accepted: 01 July 2026

Correspondence to:

Kianoosh Gholami

Anesthesiology Resident, Department of Anesthesiology, AJA University of Medical Science, Tehran, Iran

kianooshg@outlook.com

Introduction

Chronic stress is a common problem in the world that occurs in response to long and distressing situations.[1] It constitutes a generalized systemic reaction to persistent internal and external stressors and may cause psychological and biological disturbances by affecting the endocrine system, immune responses, physiological processes, and behaviors.[2,3]

Some factors influencing male infertility include genetic, hormonal, environmental, lifestyle, and psychological factors.[4] Experimental studies have demonstrated that prolonged stress exposure like CUS can cause reduced testosterone concentration, seminiferous tubule degeneration, increase germ-cell apoptosis, impair spermatogenesis, and decreased sexual behavior.[5-7] CUS is one of the validated experimental models of prolonged psychological stress, and it is commonly employed to induce anxiety or depression in rodents, which reproduce many neuroendocrine and physiological changes observed in humans.[8] Compared with a single stressor, CUS is based on several forms happening in an unpredictable order, which reduces habituation and more accurately reflects chronic stress experienced in daily life.[9]

Persistent activation of the hypothalamic–pituitary–adrenal (HPA) axis, by CUS, affects testosterone secretion by suppressing Gonadotropin-Releasing Hormone (GnRH), Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) secretion from the anterior pituitary gland.[10] Additionally, Corticotropin-Releasing Hormone (CRH) inhibits gonadotropin production as a result of chronic stress exposure that is also controlled by the hypothalamus itself.[11] Consequently, chronic psychological stress is frequently associated with reduced testosterone levels and impaired male reproductive function.[12]

Moreover, it is not established whether stress causes different reproductive system damage based on age or whether changes in PLZF expression align with age-related histopathological changes.[13] As a result, the interaction between aging and CUS on spermatogonial maintenance remains insufficiently characterized.[14]

PLZF is a transcription factor for spermatogenesis and is essential for stem cell maintenance and self-renewal activities.[15] The involvement of PLZF in chromosomal translocation with the RARαα gene is well known.[11] Decreased PLZF expression is associated with decreased spermatogonial stem cells, impaired spermatogenesis, and male infertility.[16] Therefore, PLZF appears to be a valuable molecular marker for detecting early stress-induced changes in spermatogonial stem cells.[15]

Although chronic stress is known to decrease male reproductive function it remains unclear whether stress-induced reproductive damage differs with age or whether alterations in PLZF expression parallel age-related histopathological change.[7,13] Therefore, the interaction between aging and CUS in regulating endocrine function, spermatogonial stem cell maintenance, and testicular histology remains insufficiently characterized.[7,14,17] Understanding these age-dependent effects may improve our knowledge of stress-related infertility throughout the reproductive lifespan.[17]

Accordingly, the present study evaluated the effects of CUS on blood testosterone levels, PLZF expression, and histological changes on male reproductive organs in young and old rats.

Method

Animal and Experimental design

The experiment was performed on 32 Wistar rats obtained from the Razi Institute. The rats were divided into two age groups: young rats (8 weeks old, weighing 160 ± 30 gr) and old rats (52 weeks old, weighing 400 ± 50 gr). Eight-week-old rats are considered young adults with optimal reproductive capability, while 52-week-old rats are past their reproductive peak, demonstrating natural age-related changes in testicular function and hormone levels. This division allows for the evaluation of stress effects across the reproductive span. Each age group was divided into two groups: control and stress, resulting in four groups:

1. Young Control group
2. Young Stress group
3. Old Control group
4. Old Stress group

Each group consisted of 8 rats. We selected 8 animals per group based on CUS studies that demonstrated statistically significant differences with comparable sample sizes. To prevent bias, rats were assigned to groups randomly using a computer-generated randomization sequence before starting the CUS protocol. We conducted the experiment over 8 weeks.

The adaptation progress

Rats underwent 7-day acclimatization under standardized conditions. Throughout this period, they had unrestricted access to food and water, were housed at 24 °C under a 12-hour light/12-hour dark cycle, and were observed each day for changes in body weight, overall behavior, and obvious stress indicators such as excessive grooming or decreased activity, confirming sufficient acclimatization before random assignment.

CUS Induction

After the stabilization period, we used the conventional method of CUS to induce stress, which is one of the methods that creates a similar model to depression in humans for rats.[18-20] Each day between 8:30 a.m. and 12:30 p.m. for eight weeks, the rats were randomly assigned to experience a distinct stressor, with only one stressor administered per day. Control groups did not undergo any stress procedures. Table 1 details the specific stressors and days of the experiment used in this study.

Evaluation Indicators, Procedures, and Anesthesia

After the last experiment, rats were anesthetized using a combination of ketamine (75–100 mg/kg) and Xylazine (5–10 mg/kg) administered via intraperitoneal injection 10 minutes after the last stressor. The following evaluations were performed:

1. Examination of structural changes in the testis using H&E staining.
2. IHC analysis for PLZF in testicular tissue.
3. ELISA assay for plasma testosterone concentration.

Table 1. Schedule of chronic unpredictable stressors administered during the eight-week experimental period

Week	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	-	-	PET	BSTC	CiR	BAC	8 GSC
2	MS	darkness	ISC	ISC	ISC	FS	WD
3	FL	restrainer	temperature of 45 °	FD	-	8 GSC	PET
4	MS	CiR	darkness	ISC	ISC	ISC	BAC
5	WD	restrainer	FS	temperature of 45 °	FD	FL	8 GSC
6	ISC	ISC	ISC	darkness	-	PET	CiR
7	BAC	MS	FS	restrainer	8 GSC	FD	temperature of 45 °
8	darkness	MS	ISC	ISC	ISC	-	FL
9	PET	CiR	Day of Sacrifice	-	-	-	-

24 hours food deprivation (FD), 24 hours water deprivation (WD), Bend the animal cage at a 45-degree angle for 24 hours (BAC), 1 pinch at 1 cm end of the tail (PET) 4 hours in the restrainer (restrainer), 5 minutes at a temperature of 45 ° C (temperature of 45 °), 5 minutes forced swimming in 4 ° C water (FS), 24-hour exposure of the animal to light (FL), Darkness 3 hours a day (darkness), Isolation in a small cage (three days) (ISC), Gather the animals in a small cage for 12 hours (GSC), Moisten sawdust under the animal for 24 hours (MS), Change under animal instead of rigid for 24 hours (CiR), The base stress of a tall column that was placed on a platform for 20 minutes at a height of 100 cm from the ground (BSTC).

Surgical Process and Sampling

Following the anesthesia, bilateral orchiectomy was performed through a midline scrotal incision.and the testicular tissue biopsies were obtained for fixation and tissue passage.

Investigation of testicular tissue changes in H&E staining

Testicular tissue samples were rinsed with phosphate-buffered saline (PBS) and then preserved in a 10% formaldehyde solution. Several ethanol concentrations were used for the specimens' dehydration. At the next step, we used xylene for clarification. To make tissue incisions, we impregnated the samples with paraffin. Then we made 3 µm-thick incisions on the slides using a rotary microtome. After preparation, the slides were stained with H&E and subsequently examined under a light microscope.

Measurement of serum testosterone

The blood samples were obtained from the rats after anesthesia and the surface plasma contents were extracted. Serum testosterone concentrations were measured using the ELISA kit (Ideal Tashkhis Atieh, Iran; Cat. No. TST96).

IHC analysis for PLZF

The tissues were cut to a thickness of 2µm and stored on a positively charged slide at 37 °C for 24 hours. The specimens were then degreased and dehydrated using xylene, ethanol, and PBS. The Rabbit anti-PLZF primary antibody (Abcam, United Kingdom; Cat. No. ab305064; dilution 1:200) was diluted in a suitable buffer and applied to the sections, then incubated at 4 °C for four days. After removing the primary PLZF antibody, the FITC-conjugated goat anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology, Inc; Cat. No. sc2357; dilution 1:500) was added and incubated at the same dilution for an hour. Tissues were examined under a fluorescence microscope following propidium iodide staining of the nuclei.[21] PLZF immunostaining specifically was used to identify the population of PLZF-positive undifferentiated spermatogonia (spermatogonial stem cell - SSC). This analysis therefore focuses on the SSC niche rather than differentiated germ-cell population.

Image preparation and analysis

Images were captured using an advanced color Moticam 2300 camera (Motic Inc, China) at a resolution of 1,352×1,016 pixels under standard lighting conditions at 200x magnification. PLZF-positive cells located along the basal membrane of seminiferous tubules were counted using ImageJ v1.47 software. Representative images from each experimental group were captured under identical microscopic conditions at ×200 magnification. The selected images illustrate the typical IHC staining pattern for each group and were used only for qualitative presentation rather than quantitative evaluation. PLZF-positive spermatogonial cells were expressed as a percentage of total spermatogonial population within each analyzed field.

Statistical method

Statistical analyses were conducted using SPSS version 20. Data were analyzed via two-way ANOVA followed by Bonferroni post hoc tests where appropriate, with statistical significance defined as $p < 0.05$. All results are presented as mean ± standard deviation (SD).

Ethical Approval

All experimental procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals (2007) and complied with institutional guidelines approved by the Ethics Committee of Aja University of Medical Sciences (approval ID: IR.AJAUMS.REC.1397.021).

Large-language Method Assistance

An open-weight large language model was used solely for grammar checking and revising phrasing in specific sections. No results, data analyses, statistical calculations, or scientific interpretations were generated or changed by the model. All LLM-generated text was carefully checked, verified, and edited by the authors to ensure academic accuracy.

Result

Table 2 summarizes plasma testosterone concentrations in all groups. Plasma testosterone concentrations were significantly reduced in both stress groups compared to their specific control groups ($p < 0.001$). The reduction in testosterone concentration was greater in young stressed rats than in old stressed rats.

Table 3 presents the percentage of PLZF-positive cells identified by IHC. The percentage of PLZF-positive spermatogonial cells was significantly lower in both stress groups than in their specific control groups ($p < 0.001$). While stress reduced PLZF expression in both

Table 2. The changes in plasma testosterone in the designated groups after the experiment

Plasma Testosterone	Control (ng/mL)	Stress (ng/mL)	p-value
Young	302.5±21.0	197.0±31.7	p< 0.001
Old	236.1±34.5	194.9±35.2	

*Values are presented as mean ± SD.

Table 3. The percentage of PLZF-expressing cells in the designated groups after the experiment

Tissue PLZF	Control	Stress	p-value
Young	88.5±2.8	71.7±6.6	P= 0.000
Old	43.7±2.8	26.5±1.2	

stress groups, the young stress group showed a higher decrease in PLZF-positive cells in comparison with the old stress group.

Figure 1 presents representative immunohistochemical staining for PLZF in each experimental group. PLZF-positive spermatogonial cells were primarily detected along the basal layer of the seminiferous tubules. Histological observations were consistent with the quantitative data, demonstrating a reduction in PLZF-positive cells following CUS, with the lowest immunoreactivity observed in the old stress group.

H&E staining sections demonstrated a decrease in spermatogonia in both stress groups compared to the controls (Figure 2). Only the old stress group exhibited sloughing of the seminiferous epithelium.

Discussion

In the current study, CUS significantly decreased serum testosterone levels in both young and old rats. This finding supports the previous reports indicating that chronic stress disrupts testicular steroidogenesis by activating the HPA axis and inhibiting the hypothalamic–

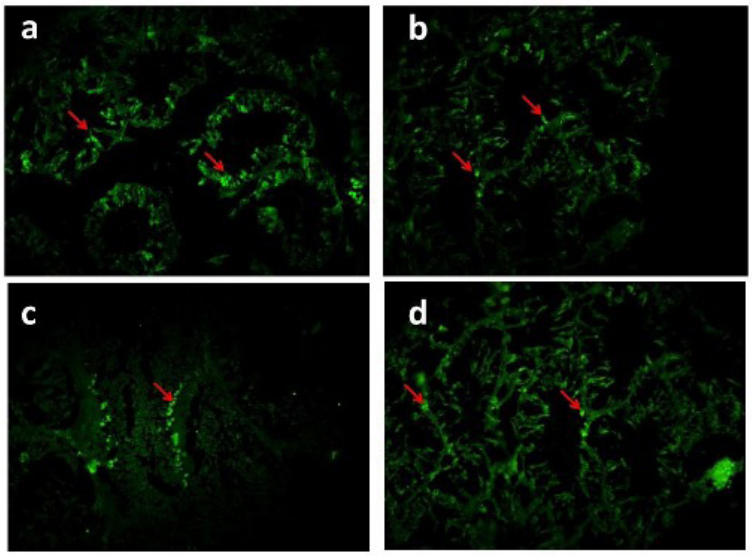

Figure 1: Representative IHC staining for PLZF in testicular tissue

Representative IHC staining for PLZF in testicular tissue from (a) young control, (b) old control, (c) young stress, and (d) old stress rats (original magnification ×200). PLZF-positive spermatogonial cells (red arrows) are located along the basal membrane of the seminiferous tubules. Representative images show reduced PLZF immunoreactivity in both stress groups compared with their respective controls, with the greatest reduction observed in the old stress group. These images are representative of each experimental group; quantitative analysis of all animals is presented in Table 3.

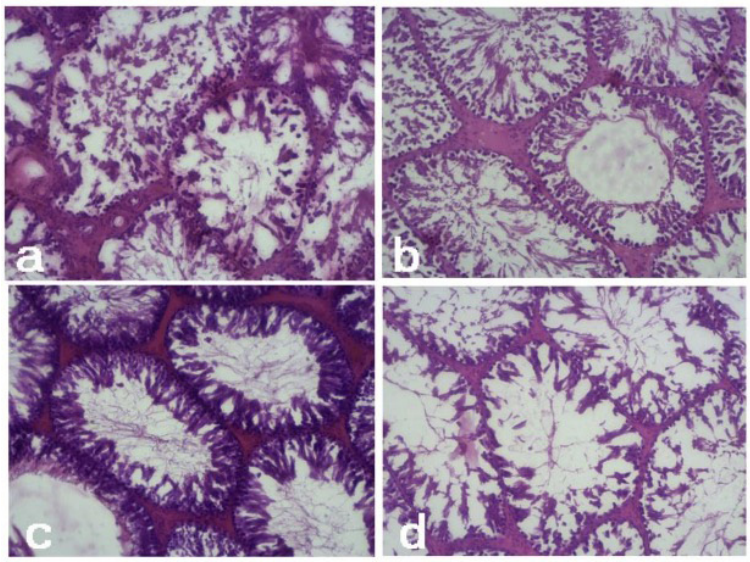

Figure 2: Representative H&E sections of testicular tissue

Representative H&E sections of testicular tissue from (A) young control, (B) old control, (C) young stress, and (D) old stress groups following eight weeks of CUS. Reduced spermatogonial cell population was observed in both stress groups, whereas seminiferous epithelial sloughing was observed only in the old stress group.

pituitary–gonadal (HPG) axis function.[22,23] Elevated glucocorticoid concentrations suppress GnRH secretion, thereby reducing LH-mediated stimulation of Leydig cells and limiting testosterone synthesis.[24,25]

The inhibitory effect of glucocorticoids on testosterone production is mediated through multiple mechanisms. Experimental studies have shown that glucocorticoids downregulate the expression of key steroidogenic proteins and enzymes, including steroidogenic acute regulatory protein (StAR), CYP11A1, HSD3B, CYP17A1, and HSD17B, enzymes that play essential roles in testosterone synthesis.[26] In addition, chronic glucocorticoid exposure may reduce Leydig-cell number through apoptosis and impair cellular proliferation, resulting in a decline in testosterone synthesis.[27]

Previous studies have shown that chronic stress impairs reproductive function through activation of the HPA axis. Increased CRH suppresses GnRH secretion and reduces pituitary LH release. In addition, the expression of CRH receptors in Leydig cells provides evidence for a direct inhibitory effect of stress signaling on testosterone synthesis. These mechanisms suggest that the reduction in testosterone is the result of endocrine dysfunction involving the HPA–HPG axis[24].

Following our study, the young stressed group had significantly lower plasma testosterone levels than older stressed group. Furthermore, the relative reduction in testosterone following CUS was greater in young rats than the older rats. This may reflect the lower baseline testosterone levels associated with reproductive aging, which limit the additional decline under stress. This explanation is supported by studies demonstrating progressive impairment of Leydig-cell function and androgen synthesis with advancing age.[25]

Stress exposure significantly reduced the PLZF expression in testicular tissue in both old and young rats, with a more significant reduction in the young group. As a key transcription factor that regulates the self-renewal and maintenance of the undifferentiated state of SSCs, PLZF plays a central role in normal spermatogenesis. Therefore, reduced expression may impair long-term spermatogenic capacity by disrupting the SSC niche.[15] These findings support the previous studies linking altered PLZF signaling to spermatogonial stem cell depletion and impaired fertility.

Importantly, the decrease in PLZF-positive cells (Figure 1) closely aligned the histological finding of reduced spermatogonial population. This concordance between molecular and histological findings suggests that chronic stress disrupts SSC maintenance and germ-cell survival, rather than causing isolated alterations in protein expression.

The testes produce sperm from spermatogonial cells during mitosis and meiosis division. This process demands the use of suitable environments. Any disruption in this procedure can result in inappropriate sperm production and infertility.[25]

Structural degeneration of the seminiferous tubules is a recognized feature of impaired spermatogenesis. The greater structural damage observed in old rats may result from diminished regenerative capacity, increased oxidative stress, and reduced tolerance to chronic endocrine disturbances.[25] Our study indicated a decrease in the spermatogonia population in both young and old groups. Also, in the old stress group, the seminiferous tubules showed sloughing. Male fertility relies on sufficient sperm production in the seminiferous epithelium.

One of the main strengths of this study is the combined evaluation of endocrine, molecular, and histopathological outcomes in young and old rats. Although the negative effects of CUS on male reproduction are well established, comparative studies examining young and old rats using PLZF as an indicator of SSC maintenance remain limited. These findings offer new insight into age-dependent responses to CUS and reproductive aging.

Conclusion

The findings of this study show that CUS affects the male reproductive system differently depending on age. Stressed young rats experienced a much greater drop in testosterone levels compared to older stressed rats. Similarly, PLZF-positive cells in testicular tissue decreased more sharply in young rats. Histological examination also showed decreased spermatogonial population in both groups, even though the seminiferous tubule damage was only detected in the older stressed rats. Our results suggest that young males may be more influenced by the negative effects of CUS, including declined testosterone levels and reduced spermatogenesis.

Acknowledgement

Our sincere gratitude goes out to animal lab personnel and guard units who cooperated with us.

Conflict of Interest

There are no conflicts of interest between the authors of this article in terms of funding, research support, consultation fees, patents, or financial interests. Additionally, there are no conflicts of interest, such as unpaid membership in a government or non-governmental organization.

Source of Funding

This study did not receive any external funding.

Ethical Considerations

The authors of this study have adhered strictly to ethical issues (such as plagiarism and data fabrication, as well as double publication). Ethics approval was also received from Aja University of Medical Sciences and the ethics committee (IR.AJAUMS.REC.1397.021).

References

1. Shi X, Wu J. Chronic stress and anticipatory event-related potentials: the moderating role of resilience. Stress. 2020;23(5):607–13. doi:10.1080/10253890.2020.1766019
2. Yao BC, Meng LB, Hao ML, Zhang YM, Gong T, Guo ZG. Chronic stress: a critical risk factor for atherosclerosis. J Int Med Res. 2019;47(4):1429–40. doi:10.1177/0300060519826
3. de OC, de OCM, IC M, AS Q, PRM F, de SFR. Hypercaloric diet modulates effects of chronic stress: a behavioral and biometric study on rats. Stress. 2015;18(5):514–23. doi:10.3109/10253890.2015.1079616
4. Okonofua FE, Ntoimo LFC, Omonkhua A, Ayodeji O, Olafusi C, Unuabonah E, et al. Causes and Risk Factors for Male Infertility: A Scoping Review of Published Studies. Int J Gen Med. 2022 Jul;Volume 15:5985–97. doi:10.2147/IJGM.S363959
5. Odetayo AF, Akhigbe RE, Bassey GE, Hamed MA, Olayaki LA. Impact of stress on male fertility: role of gonadotropin inhibitory hormone. Front Endocrinol Lausanne. 2024;14(1329564). doi:10.3389/fendo.2023.1329564
6. Yadav S, Yadav A, Mishra RK. Chronic unpredictable stress exposure disrupts testicular function by modulating germ cell-junctional dynamics and Nrf2/HO-1/IKKβ/NF-κB pathway. Reprod Toxicol. 2025;132(108845). doi:10.1016/j.reprotox.2025.108845
7. Tong LI, Jiwei YA, Qianwei LI, Jingcheng LI, Xiujuan WA, Weibing LI, et al. Chronic stress impairs male spermatogenesis function and Nectin-3 protein expression in the testis. Physiol Res. 2020;23;69(2):297. doi:10.33549/physiolres.[934287]
8. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. Neurosci Biobehav Rev. 2019;99:101–16. doi:10.1016/j.neubiorev.2018.12.002
9. Sequeira-Cordero A, Salas-Bastos A, Fornaguera J, Brenes JC. Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. Sci Rep. 2019 Nov 22;9(1):17403. doi:10.1038/s41598-019-53624-1
10. Erdemir F, Atilgan D, Firat F, Markoc F, Parlaktas BS, Sogut E. The effect of sertraline, paroxetine, fluoxetine and escitalopram on testicular tissue and oxidative stress parameters in rats. Int Braz J Urol. 2014;40(1):100–8. doi:10.1590/S1677-5538.IBJU.2014.01.15
11. Hussain L, Maimaitiyiming Y, Islam K, Naranmandura H. Acute promyelocytic leukemia and variant fusion proteins: PLZF-RARα fusion protein at a glance. Semin Oncol. 2019 Apr;46(2):133–44. doi:10.1053/j.seminoncol.2019.04.004
12. Rastrelli G, Corona G, Maggi M. Testosterone and sexual function in men. Maturitas. 2018;112:46–52. doi:10.1016/j.maturitas.2018.04.004

13. Ferder IC, Wang N. Hypermaintenance and hypofunction of aged spermatogonia: insight from age-related increase of Plzf expression. Oncotarget. 2015 Jun 30;6(18):15891–901. doi:10.18632/oncotarget.[4045]

14. Yadav S, Yadav A, Mishra RK. Chronic unpredictable stress exposure disrupts testicular function by modulating germ cell-junctional dynamics and Nrf2/HO-1/IKKβ/NF-κB pathway. Reprod Toxicol. 2025 Mar;132:108845. doi:10.1016/j.reprotox.2025.108845

15. Ghasemi N, Azizi H, Razavi-Amoli SK, Skutella T. The Role of Plzf in Spermatogonial Stem Cell Maintenance and Differentiation: Mapping the Transcriptional Dynamics and Key Interactions. Cells. 2024 Nov 21;13(23):1930. doi:10.3390/cells13231930

16. Azizi H, Koruji M, Skutella T. Comparison of PLZF gene expression between pluripotent stem cells and testicular germ cells. Cell J. 2020;22(1):60–5. doi:10.22074/cellj.2020.6532

17. Matzkin ME, Calandra RS, Rossi SP, Bartke A, Frungieri MB. Hallmarks of Testicular Aging: The Challenge of Anti-Inflammatory and Antioxidant Therapies Using Natural and/or Pharmacological Compounds to Improve the Physiopathological Status of the Aged Male Gonad. Cells. 2021 Nov 10;10(11):3114. doi:10.3390/cells10113114

18. Harro J, Häidkind R, Harro M, Modiri AR, Gillberg PG, Pähkla R, et al. Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. Eur Neuropsychopharmacol. 1999 Dec;1;10(1):5-16.

19. Black PH. The inflammatory response is an integral part of the stress response: implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. Brain Behav Immun. 2003;17(5):350–64.

20. AMIHAESEI IC, Chelaru L. Metabolic syndrome: a widespread threatening condition; risk factors, diagnostic criteria, therapeutic options, prevention and controversies: an overview. Med Surg J. 2014;118(4):896–900.

21. Campbell SC, Macfarlane WM. Detection of insulin production by immunohistochemistry. In: Diabetes Mellitus. Springer; 2003. p. 47–9.

22. Mbiydzenyuy NE, Qulu LA. Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metab Brain Dis. 2024 Jul 31;39(8):1613–36. doi:10.1007/s11011-024-01393-w

23. Tian P, Lv P, Shi W, Zhu M, Cong B, Wen B. Chronic stress reduces spermatogenic cell proliferation in rat testis. Int J Clin Exp Pathol. 2019;12(5):1921–6.

24. Domes G, Linnig K, Von Dawans B. Gonads under stress: A systematic review and meta-analysis on the effects of acute psychosocial stress on gonadal steroids secretion in humans. Psychoneuroendocrinology. 2024 Jun;164:107004. doi:10.1016/j.psyneuen.2024.107004

25. Kaltsas A, Dimitriadis F, Zachariou A, Koukos S, Chrisofos M, Sofikitis N. When Testosterone Fades: Leydig Cell Aging Shaped by Environmental Toxicants, Metabolic Dysfunction, and Testicular Niche Crosstalk. Cells. 2026 Jan 15;15(2):158. doi:10.3390/cells15020158

26. Valdez R, Cavinder CA, Varner DD, Welsh TH, Vogelsang MM, Ing NH. Dexamethasone downregulates expression of several genes encoding orphan nuclear receptors that are important to steroidogenesis in stallion testes. J Biochem Mol Toxicol. 2019 Jun;33(6):e22309. doi:10.1002/jbt.[22309]

27. Gao HB, Tong MH, Hu YQ, Guo QS, Ge R, Hardy MP. Glucocorticoid Induces Apoptosis in Rat Leydig Cells. Endocrinology. 2002 Jan 1;143(1):130–8. doi:10.1210/endo.143.1.8604

Supplementary Files

Age-Dependent Effects of Chronic Unpredictable Stress on Male Reproductive Organs in Rats

Appendix 1. Representative IHC staining for PLZF in testicular tissue

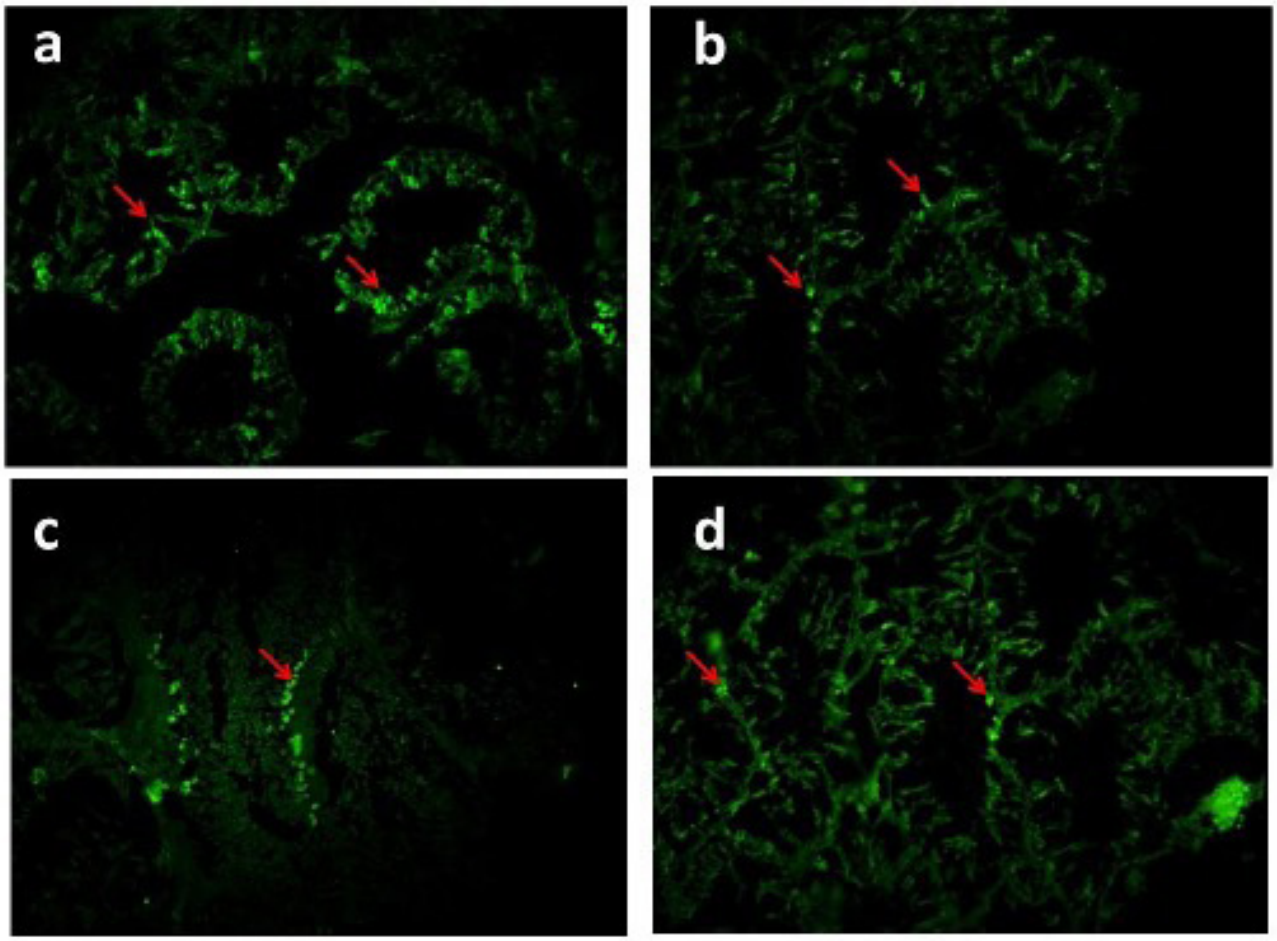

Figure 1: Representative IHC staining for PLZF in testicular tissue

Representative IHC staining for PLZF in testicular tissue from (a) young control, (b) old control, (c) young stress, and (d) old stress rats (original magnification ×200). PLZF-positive spermatogonial cells (red arrows) are located along the basal membrane of the seminiferous tubules. Representative images show reduced PLZF immunoreactivity in both stress groups compared with their respective controls, with the greatest reduction observed in the old stress group. These images are representative of each experimental group; quantitative analysis of all animals is presented in Table 3.

Appendix 2. Representative H&E sections of testicular tissue

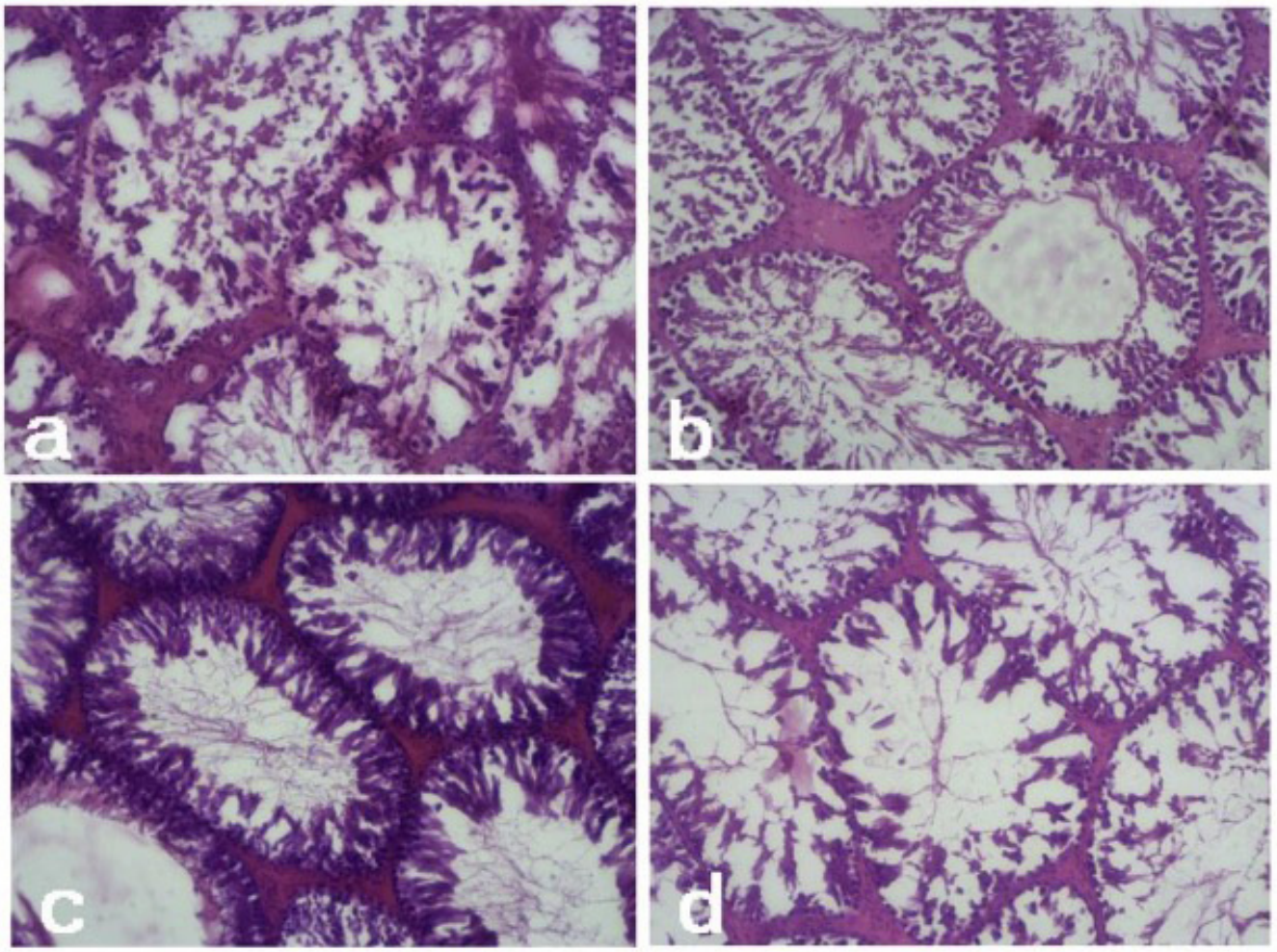

Figure 2: Representative H&E sections of testicular tissue

Representative H&E sections of testicular tissue from (A) young control, (B) old control, (C) young stress, and (D) old stress groups following eight weeks of CUS. Reduced spermatogonial cell population was observed in both stress groups, whereas seminiferous epithelial sloughing was observed only in the old stress group.

Appendix 3. Schedule of chronic unpredictable stressors administered during the eight-week experimental period

Week	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
1	-	-	PET	BSTC	CiR	BAC	8 GSC
2	MS	darkness	ISC	ISC	ISC	FS	WD
3	FL	restrainer	temperature of 45 °	FD	-	8 GSC	PET
4	MS	CiR	darkness	ISC	ISC	ISC	BAC
5	WD	restrainer	FS	temperature of 45 °	FD	FL	8 GSC
6	ISC	ISC	ISC	darkness	-	PET	CiR
7	BAC	MS	FS	restrainer	8 GSC	FD	temperature of 45 °
8	darkness	MS	ISC	ISC	ISC	-	FL
9	PET	CiR	Day of Sacrifice	-	-	-	-

24 hours food deprivation (FD), 24 hours water deprivation (WD), Bend the animal cage at a 45-degree angle for 24 hours (BAC), 1 pinch at 1 cm end of the tail (PET) 4 hours in the restrainer (restrainer), 5 minutes at a temperature of 45 ° C (temperature of 45 °), 5 minutes forced swimming in 4 ° C water (FS), 24-hour exposure of the animal to light (FL), Darkness 3 hours a day (darkness), Isolation in a small cage (three days) (ISC), Gather the animals in a small cage for 12 hours (GSC), Moisten sawdust under the animal for 24 hours (MS), Change under animal instead of rigid for 24 hours (CiR), The base stress of a tall column that was placed on a platform for 20 minutes at a height of 100 cm from the ground (BSTC).

Appendix 4. The changes in plasma testosterone in the designated groups after the experiment

Plasma Testosterone	Control (ng/mL)	Stress (ng/mL)	p-value
Young	302.5±21.0	197.0±31.7	p< 0.001
Old	236.1±34.5	194.9±35.2	

*Values are presented as mean ± SD.

Appendix 5. The percentage of PLZF-expressing cells in the designated groups after the experiment

Tissue PLZF	Control	Stress	p-value
Young	88.5±2.8	71.7±6.6	P= 0.000
Old	43.7±2.8	26.5±1.2	

DOI: 10.52629/jamsa.v12i1.1119

Comparative Efficacy and Safety of Dual Orexin Receptor Antagonists (DORAs) Versus Z-Drugs for Insomnia in Older Adults : A Systematic Review

Background: Insomnia affects up to 48% of adults ≥55 globally, often treated with Z-drugs which may increase incidence of cognitive impairment and adverse events such as falls. Dual Orexin Receptor Antagonists (DORAs) offer a targeted alternative by specifically suppressing wakefulness-promoting pathways to minimize these adverse effects. Therefore, this study aims to compare the effectiveness and safety profile of DORAs and Z-Drugs.

Objective: This systematic review aims to compare the efficacy and safety of Dual Orexin Receptor Antagonists (DORAs) against Z-drugs as an alternative therapy for diagnosed insomnia present in older adults.

Methods: The PRISMA 2020 standards were followed in conducting this systematic review. RCTs were searched through Online databases including PubMed, ScienceDirect, ProQuest and Sage Journals. The review included studies on adults aged ≥55 years with diagnosed insomnia with DORAs and Z-Drugs as the interventions. The studies recovered were then assessed by the Cochrane RoB 2 risk of bias tool. Adverse events were assessed as the primary outcome; SOL, WASO and overall sleep quality improvement as the secondary outcome.

Results: Seven studies consisting of RCT, Crossover and SUNRISE-1 Cohort were evaluated. DORAs demonstrated significantly superior efficacy over Z-drugs, with Least Squares Mean differences of -6.7 and -8.0 minutes for 5 mg and 10 mg doses, respectively. Furthermore, safety analyses revealed DORAs had a significantly lower relative incidence of residual effects and cognitive impairment (<5%) compared to Z-drugs (>15%).

Conclusion: Although DORAs and Z-Drugs are used to manage insomnia, DORAs appeared to have lower side effects and lower levels of falls during the intervention. However further quantitative analysis is needed to confirm its efficacy and heterogeneity in the studies limits confidence levels in current findings.

Keywords: Insomnia, Dual Orexin Receptor Antagonists, Z-Drugs, Accidental Falls, Older Adults

Jason Conrad Tanely[1], Frederick Karel Hilmawan[1], Given Anugraha Wijaya[1]

[1]Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

Category: Review Articles

Date Received: 30 May 2026

Date Accepted: 29 June 2026

Correspondence to:

Jason Conrad Tanely

Atma Jaya Catholic University of Indonesia

jasonct200831@gmail.com

DOI: 10.52629/jamsa.v12i1.1119

Introduction

Insomnia is one of the most common sleep disorders suffered by the elderly population and these situations are affecting growing public health concern worldwide. Physiological changes such as pattern in circadian rhythm, reduced sleep efficiency, and shorter sleep duration, making adults aged over ≥55 years particularly vulnerable to insomnia. Studies estimated that approximately 30-48% of older adults worldwide are experiencing symptoms of insomnia, whereas specifically 12-20% of the elderly individual meet the criteria.[1,2] Prolonged insomnia that occurs in the elderly population is associated with impaired daily daytime functional performance, reduced quality of life, depression, and decline in their cognitive performance.[3] Moreover, insomnia significantly increases the risk of falls, fracture, and accidents which are the major contributors to morbidity and mortality among the geriatric populations.[4]

In Indonesia, reports from several national and regional studies show that insomnia symptoms are affecting 30.3% of the Indonesia adults population, particularly among individuals aged ≥55 years.[5] The prevalence tends to increase with age and is associated with chronic disease suffered among the older adults. Despite its high prevalence, insomnia remains undertreated in Indonesia due to low awareness and low exposure of sleep health education.[5] Consequently, pharmacological therapies are often utilised as the primary treatment for symptoms among older patients.

Historically, non-benzodiazepine hypnotics commonly referred to as Z-drugs (such as zolpidem and zopiclone) have been widely used for the pharmacological treatment of insomnia.[6] While effective in initiating and maintaining sleep by enhancing GABAergic inhibition, their use in the older adults is frequently complicated by adverse effects, including cognitive impairment, psychomotor depression, and a significantly increased risk of falls.[7]

Dual Orexin Receptor Antagonists (DORAs) has recently gained popularity emerging as a novel therapeutic class.[8] Unlike Z-drugs, which broadly depress the central nervous system, DORAs (such as lemborexant and suvorexant) target the orexin neuropeptide system to specifically block wakefulness-promoting pathways. This targeted mechanism presents a potentially safer side-effect profile for older adults, mitigating many of the classical hypnotic risks and still effectively reducing insomnia for older adults. When selecting hypnotic therapy it is important to consider fall-related safety in the context of insomnia and sleep disturbance.[9] Recent evidence suggests that orexin receptor antagonists do not significantly increase the risk of falls or fractures in patients treated for insomnia, although available data for DORAs remain limited. In addition, lemborexant has been shown to improve sleep variables in older adults without impairing cognitive function, risk of falls or fractures compared with placebo. These findings demonstrate that DORAs may offer a safer therapeutic profile than traditional non-benzodiazepine hypnotics for older adults with insomnia and sleep disturbance.[9,13,17] However, direct comparative evidence between DORAs and Z-drugs in adults aged ≥55 years remains limited, especially regarding important safety outcomes such as risk of falls, morning somnolence, and cognitive or psychomotor impairment. Therefore, this review aims to evaluate the safety and efficacy of DORAs compared with Z-drugs in adults aged ≥55 years with primary or comorbid insomnia.

Method

PICO

This systematic review was conducted using the PICO approach (Population, Intervention, Comparison and Outcome).[10] In this study, the population included were older adults aged ≥55 years with formally diagnosed insomnia. The main interventions used were Dual Orexin Receptor Antagonists (DORAs), while Z-drugs and placebo were used as a comparison. The primary outcome assessed were the incidence of adverse events. The Secondary outcomes were Sleep onset latency (SOL), wake after sleep onset (WASO), and overall sleep quality improvement.

Search Strategy

A comprehensive literature search was systematically conducted across four electronic databases: PubMed, ScienceDirect, ProQuest, and Sage Journals, from May 12, 2026, to May 28, 2026. The search process used a combination of keywords that are relevant to this review. Identified studies are then screened using Rayyan.ai as a tool for selecting studies based on the inclusion and exclusion criteria. To ensure rigorous methodology in accordance with PRISMA guidelines[11], the study selection and data extraction processes were independently conducted by two reviewers (J.C.T. & F.K.H.). Any disagreements regarding study eligibility during the screening process were resolved through a

DOI: 10.52629/jamsa.v12i1.1119

Table 1. PICO Framework

Population	Intervention	Comparison	Comparison
Older adult patients (aged ≥50 years) formally diagnosed with primary insomnia or insomnia comorbid with other conditions.	Dual Orexin Receptor Antagonists (Lemborexant, Daridorexant and Suvorexant)	Traditional non-benzodiazepine hypnotics (Z-drugs), including agents such as Zolpidem, Zopiclone	• Primary: Incidence of adverse events (specifically focusing on falls, morning hangover/ somnolence, and next-morning cognitive/ psychomotor impairment). • Secondary: Sleep onset latency (SOL), wake after sleep onset (WASO), and overall sleep quality improvement

joint full-text re-evaluation to reach a mutual consensus. A third reviewer from the research team was available for final arbitration if a consensus could not be achieved. The search strategy was developed using five core keywords: Insomnia, Dual Orexin Receptor Antagonists, Z-Drugs, Accidental Falls, Older Adults

The inclusion criteria for this manuscript were visualized using the PRISMA guideline.[11] Eligible study designs included studies of Randomized Controlled Trials (RCT) and high quality observational studies such as prospective or retrospective cohort studies. The studies involved older adults aged ≥ 55 years which have a higher risk of insomnia due to decreased quality and quantity of sleep as the brain ages, DORAs such as Lemborexant, Suvorexant and Daridorexant used as primary therapy to treat insomnia were included in the selection process. Studies were also required to assess the incidence of adverse events such as falls, somnolence and psychomotor impairments as the primary outcomes. Alongside Sleep Onset Latency (SOL), Wake After Sleep Onset (WASO) and the overall quality of sleep as the secondary assessed outcome.

Exclusion criteria were also developed using the PRISMA guideline.[11] Studies were excluded if they met one of these criteria. Systematic reviews, meta-analyses, narrative reviews, in vitro or animal studies, insomnia present in adults <50 years of age, other diagnosed sleep disorders, studies that don't use DORAs as a primary intervention, studies without a relevant comparison group such as Zolpidem or Zopiclone and studies that do not assess the overall safety and efficacy of the sleep-related outcomes.

Risk of Bias Assessment

The included studies were then assessed for risk of bias using tools tailored based on their study design. The

Table 2. Search Strategy

Database	Search Strategy	Search Results
PubMed	("Sleep Initiation and Maintenance Disorders"[Mesh] OR "insomnia"[tiab] OR "sleep disorder*"[tiab]) AND ("lemborexant"[tiab] OR "daridorexant"[tiab] OR "Dayvigo"[tiab] OR "Quviviq"[tiab]) AND ("zolpidem"[Mesh] OR "zolpidem"[tiab] OR "zopiclone"[tiab] OR "Ambien"[tiab] OR "Imovane"[tiab])	27
ScienceDirect	(insomnia OR "sleep disorder") AND (lemborexant OR daridorexant OR suvorexant) AND (zolpidem OR zopiclone)	53
Proquest	Box 1: insomnia OR "sleep disorder" OR "sleep disorders" Box 2 (AND): lemborexant OR daridorexant OR Dayvigo OR Quviviq Box 3 (AND): zolpidem OR zopiclone OR Ambien OR Imovane	140
Sage Journal	(insomnia OR "sleep disorder" OR "sleep disorders") AND (lemborexant OR daridorexant OR Dayvigo OR Quviviq) AND (zolpidem OR zopiclone OR Ambien OR Imovane)	15

DOI: 10.52629/jamsa.v12i1.1119

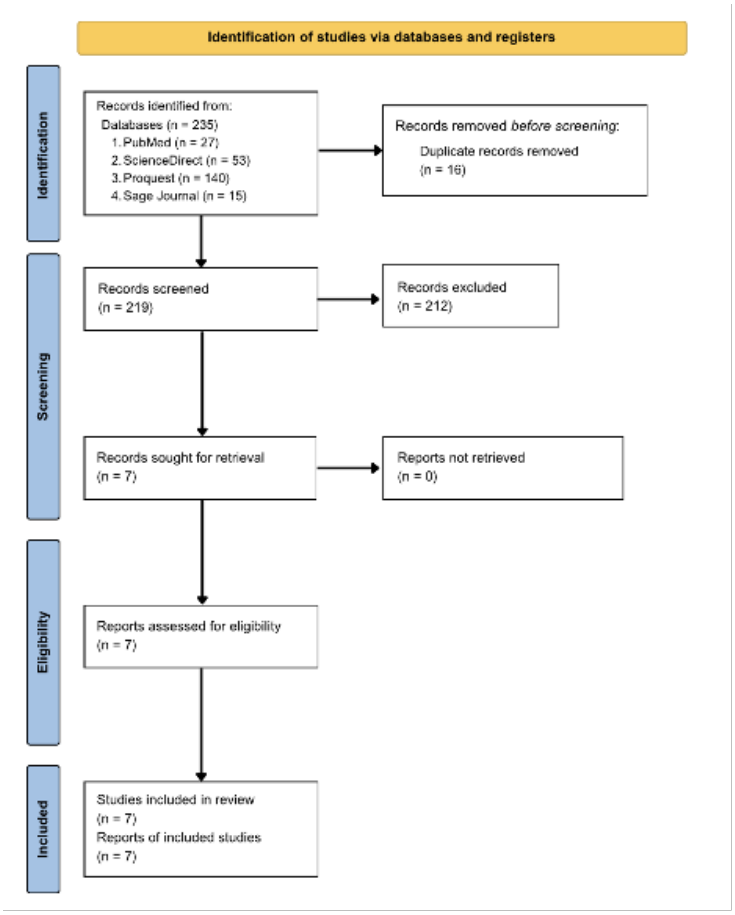

Figure 1: PRISMA Flowchart

risk of bias for Randomized Controlled Trials, SunRISe-1 trials and Crossover trials were assessed using the Cochrane Risk of Bias 2 (ROB 2.0) tool, which assesses the study based on five domains to determine the overall methodological quality.[12]

Result

A total of seven studies consisting of five parallel randomized controlled trials and two randomized crossover trials were evaluated in this review (Table 3). Due to the high clinical and methodological heterogeneity across the included studies, particularly the variations in study design and the diverse measurement tools used for primary and secondary outcomes, a quantitative meta-analysis was not feasible. Consequently, a narrative synthesis approach was employed to compare the findings across the studies.

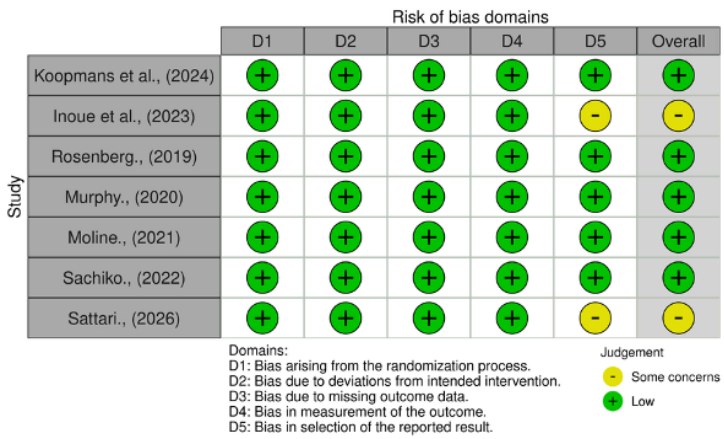

Figure 2: Risk of Bias Traffic Plot

DOI: 10.52629/jamsa.v12i1.1119

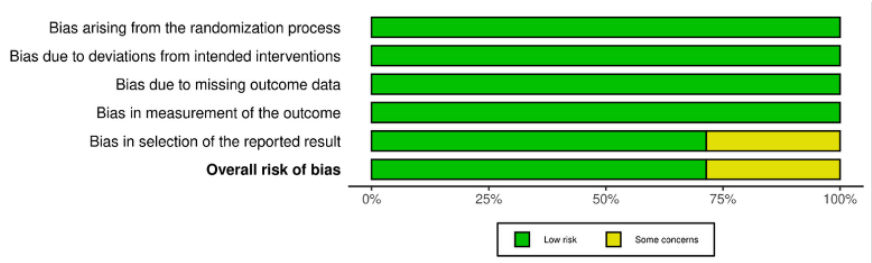

Figure 3: Risk of Bias Conclusion

Study quality appraisal conducted using the Cochrane ROB 2.0 tool across five domains: bias arising from the randomization process (D1), bias due to deviations from intended intervention (D2), bias due to missing outcome data (D3), bias in measurement of the outcome (D4), and bias in selection of the reported result (D5). The overall quality of the studies was generally at a low risk of bias, indicating an adequate randomization process, correct interventions used, and complete outcome data across the studies. Nevertheless, the main area of concern was observed in domain five (D5) with 2 studies observed to have some concerns (n=2). Although the trials were pre-registered, the nature of the post hoc analysis showed a risk of selective reporting in the trials.

Discussion

Safety Findings (Primary Outcome)

Based on our narrative synthesis, the analysis of safety parameters illustrates distinct differences in residual effect profiles that are vital for the older adult population.[13] Quantitatively, the use of a Z-drug (zolpidem) is associated with significantly higher levels of postural instability and morning cognitive impairment, with a relative incidence of >15%. In contrast, the DORA class maintains lower incidence metrics closer to baseline or placebo levels (<5%).[14,15] These safety findings confirm that the DORA class successfully minimizes the risk of falls during the night and the following morning, directly addressing a major historical challenge in the pharmacotherapy of geriatric insomnia.

Efficacy Findings

With respect to sleep maintenance efficacy, 5 mg and 10 mg doses of lemborexant were significantly superior to 6.25 mg of zolpidem in reducing Wake After Sleep Onset in the second half of the night (WASO-2H).[16] The Least Squares Mean (LSM) differences were -6.7 minutes (95% CI: -11.2 to -2.2) for the 5 mg dose and -8.0 minutes (95% CI: -12.5 to -3.5) for the 10 mg dose.[14] In a broader clinical context, this reduction in WASO addresses a fundamental characteristic of geriatric insomnia: older adults frequently experience fragmented sleep and frequent nocturnal awakenings. While Z-drugs may aid initial sleep onset, they often fail to sustain sleep through the second half of the night.[17] In contrast, the sustained, targeted action of DORAs supports a more prolonged and uninterrupted sleep cycle, translating to more restorative sleep essential for daytime functioning and quality of life in the elderly population.[3]

Mechanistic Explanations

Following the evaluation of safety, the secondary outcomes regarding sleep maintenance efficacy highlight a clear clinical advantage for Dual Orexin Receptor Antagonists (DORAs) over conventional Z-drugs in managing geriatric insomnia. Rather than suppressing global neural activity through GABAergic hyperactivation as Zolpidem does. DORAs does inhibit the wake-promoting orexin pathway, resulting in a much more natural and physiological sleep state. The focused target mechanisms also improve sleep architecture. EEG and polysomnography data confirm that lemborexant preserves natural sleep microarchitecture over time, supported by data showing an increasing Rapid Eye Movement (REM) sleep proportion and shortening REM latency.[18] The data also suggests that a one-size-fits-all aspect is outdated. Lemborexant demonstrates more consistent objective and subjective benefits specifically for older adults with a short sleep duration (<6 hours), which proved highly effective for those struggling with maintenance insomnia.[19]

Clinical Implications

The findings of this review have substantial clinical applicability for the current landscape of geriatric care. A part of shifting away from GABAergic agents in favour towards DORAs marks a much more necessary evolution in geriatric, in whom reliable sleep maintenance must be balanced against the risk of cognitive impairment

DOI: 10.52629/jamsa.v12i1.1119

Table 3. Study Characterisation

First Author, year	Design	Population (N,Stage Age)	Intervention (I)	Comparator (C)	Protocol (Dose/ Freq)	Outcomes
Koopmans , 2024	RCT	N: 18, Stage: Healthy elderly, Age: 66 - 88 (mean: 71.9)	Suvorexant	Zolpidem and placebo	Dose: 10 mg suvorexant, 5 mg zolpidem, or placebo. Frequency: Single dose administered in the morning following a 2-hour fast	Interactive Walk Way (IWW) Body Sway Adaptive tracking (%) Timed Up and Go
Inoue , 2023	RCT	N: 1006 Stage: Participants met the DSM-5 criteria for insomnia disorder Age: ≥ 55 years	Lemborexant	Zolpidem and placebo	Single daily oral dose (5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo) for 30 nights, taken within 5 minutes of the time they intended to sleep	SOL WASO TST
Rosenberg , 2019	RCT	N: 1006 Stage: Participants met the DSM-5 criteria for insomnia disorder Age: 63.9 (Mean)	Lemborexant	Zolpidem and placebo	5 mg of lemborexant, 10 mg of lemborexant 6.25 mg of zolpidem, or placebo in a 5:5:5:4 ratio.	Latency to persistent sleep WASO Sleep efficiency (%) AEs
Murphy , 2020	CrossOver	Healthy Older Adults Women ≥ 55 y.o., Men ≥ 65 y.o. (Crossover design)	Lemborexant 5 mg and 10 mg (Single-dose, 4-period crossover)	Placebo Zolpidem ER (6.25 mg)	Dose: 5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo. Single dose at bedtime in a 4-period crossover design.	5 mg and 10 mg showed no significant impairment in MOTN postural stability or AAT compared to placebo.
Moline , 2021	SUNRISE-1 Cohort	Older Adults with Insomnia ≥ 55 y.o. (SUNRISE-1 Cohort)	Lemborexant 5 mg and 10 mg (1 month)	Placebo Zolpidem ER (6.25 mg)	Dose: 5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo. Single daily oral dose at bedtime for 1 month.	LEM significantly increased TST and REM sleep, and decreased latency to REM compared to both placebo and Zolpidem.

DOI: 10.52629/jamsa.v12i1.1119

First Author, year	Design	Population (N,Stage Age)	Intervention (I)	Comparator (C)	Protocol (Dose/ Freq)	Outcomes
Sachiko, 2022	CrossOver	Healthy Elderly Subjects Older adults (Evaluated via crossover)	Suvorexant (Low dose) Ramelteon	Placebo Zolpidem	Dose: Suvorexant (low dose), ramelteon, zolpidem, or placebo. Single dose at bedtime in a crossover design	Low-dose Suvorexant and Ramelteon demonstrated significantly fewer next-morning residual effects compared to Zolpidem.
Sattori , 2026	RCT	Older Adults with Insomnia ≥ 55 y.o. (RCT)	Lemborexant (Acute and chronic use)	Zolpidem	Dose: Lemborexant or zolpidem. Acute and chronic administration at bedtime	ZOL & LEM different effects LEM better maintains physiological sleep microarchitecture

and nocturnal falls. In routine geriatric practice, DORAs may be particularly favorable for frail older patients, those with a history of falls, and those receiving concurrent polypharmacy, given their comparatively lower psychomotor and cognitive burden relative to Z-drugs. Nonetheless, clinical adoption should remain proportionate to the current strength of evidence, which still remains limited by heterogeneity across the available studies.

Strengths

This review itself benefits from a systematic type of plan , PRISMA-guided search strategy across four databases, independent dual-reviewer screening, and structured risk-of-bias appraisal using the Cochrane RoB 2 tool which provides a transparent and better synthesis of the current evidence. By intentionally including both randomized and high-quality non-randomized designs, this review captures a broader range of controlled and real-world evidence than a review restricted to RCTs alone, offering a better comprehensive view of patient care that connects controlled polysomnographic efficacy with real-world functional safety outcomes.

Limitations and Future Research

Despite the promising findings, this review is constrained by limitations within the current evidence base. Specifically, the clinical and methodological heterogeneity observed across the current literature prevents a formal meta-analysis and restricts the statistical data power. However, it still provides a necessary and comprehensive view of patient care that really connects the gap between controlled polysomnography efficacy and real-world functional safety. To address these evidence gaps, there is a critical need for more standardized study designs. Future research should prioritize long-term, randomized controlled trials (RCTs) that specifically stratify geriatric patients based on their frailty status and varying degrees of polypharmacy.[20] Additionally, evaluating the efficacy of DORAs in older adults with comorbid neurocognitive disorders, such as early-stage dementia or Alzheimer's disease, remains an underexplored area that is essential for translating these polysomnographic benefits into broader, real-world clinical guidelines.[21]

Conclusion

In conclusion, Dual Orexin Receptor Antagonists (DORAs), particularly lemborexant and suvorexant demonstrate superior safety profiles and favorable efficacy compared with conventional Z-drugs on managing insomnia among older adults. DORAs significantly improve sleep maintenance parameters especially Wake After Sleep Onset (WASO), while preserving REM sleep quality. In contrast with zolpidem, DORAs were proven with lower rates of next-morning

DOI: 10.52629/jamsa.v12i1.1119

residual effects, cognitive impairment, postural instability, and fall risk, which are major concerns in geriatric populations.

The therapeutic advantages of DORAs are linked to their selective inhibition of orexin-mediated wakefulness pathways, giving more natural sleep induction without excessive global central nervous system suppression seen in GABAergic hypnotics. However, given the substantial clinical and methodological heterogeneity and the relatively small evidence base identified in this review, these findings should be regarded as preliminary rather than conclusive. DORAs represent a promising and physiologically rational pharmacological option for older adults with insomnia, but confirmation through standardized, adequately powered trials is required before firm clinical recommendations can be made.

Acknowledgement

We sincerely thank the researchers and authors of the primary studies included in this systematic review for their foundational scientific contributions.

Conflict of Interest

The authors report no commercial associations, financial ties, or personal relationships that might pose a conflict of interest in connection with this manuscript.

Source of Funding

This research received no specific grant or funding from any agency in the public, commercial, or not-for-profit sectors.

References

1. Miner B, Kryger MH. Sleep in the aging population. Sleep Med Clin. 2020;15(2):311–8. doi:10.1016/j.jsmc.2020.02.016.
2. Ohayon MM, Reynolds CF 3rd. Epidemiological and clinical relevance of insomnia diagnosis algorithms according to the DSM-IV and the International Classification of Sleep Disorders (ICSD). Sleep Med. 2009;10(9):952–60. doi:10.1016/j.sleep.2009.07.008.
3. Patel D, Steinberg J, Patel P. Insomnia in the elderly: a review. J Clin Sleep Med. 2018;14(6):1017–24. doi:10.5664/jcsm.7172.
4. Kementerian Kesehatan Republik Indonesia. Gangguan tidur pada lansia [Internet]. Jakarta: Kementerian Kesehatan RI; [cited 2026 May 16]. Available from: https://keslan.kemkes.go.id/view_artikel/68/gangguan-tidur-pada-lansia
5. Rahman FF, Lin KY, Lin KH. Sleep disturbance and associated factors in older adults using data from the Indonesian Family Life Survey. Int J Gerontol. 2024;18(3):184–90. doi:10.6890/IJGE.202407_18(3).0009.
6. Amari DT, Juday T, Frech FH, et al. Falls, healthcare resources and costs in older adults with insomnia treated with zolpidem, trazodone, or benzodiazepines. BMC Geriatr. 2022;22:484. doi:10.1186/s12877-022-03165-6.
7. Treves N, Perlman A, Geron LK, Asaly A, Matok I. Z-drugs and risk for falls and fractures in older adults: a systematic review and meta-analysis. Age Ageing. 2018;47(2):201–8. doi:10.1093/ageing/afx167.
8. Young J, Lee E, Lindblad AJ, Falk J. Orexin antagonists for insomnia. Can Fam Physician. 2024;70(3):183–4. doi:10.46747/cfp.7003183.
9. Pan G, Ni L, Yan H, Yao L. Association between the use of orexin receptor antagonists and falls or fractures: a meta-analysis. J Psychiatr Res. 2024;176:393-402. doi:10.1016/j.jpsychires.2024.06.029.
10. Richardson WS, Wilson MC, Nishikawa J, Hayward RS. The well-built clinical question: a key to evidence-based decisions. ACP J Club. 1995;123(3):A12-A13.
11. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
12. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
13. Rosenberg R, Murphy P, Zammit G, Mayleben D, Kumar D, Dhadda S, et al. Comparison of Lemborexant With Placebo and Zolpidem Tartrate Extended Release for the Treatment of Older Adults With Insomnia Disorder: A Phase 3 Randomized Clinical Trial. JAMA Netw Open. 2019;2(12):e1918254.
14. Uemura SI, Imanishi A, Terui Y, Park I, Satake M, Han G, et al. Residual effects of low dose of suvorexant, zolpidem, and ramelteon in healthy elderly subjects: A randomized double-blind study. Neuropsychopharmacol Rep. 2022;42(3).
15. Moline M, Zammit G, Cheng JY, Perdomo C, Kumar D, Mayleben D. Comparison of the effect of lemborexant with placebo and zolpidem tartrate extended release on sleep architecture in older adults with insomnia disorder. J Clin Sleep Med. 2021;17(6):1167–1174.
16. Koopmans I, Geerse D, de Ridder L, Roerdink M, Juachon MJ, Muehlan C, et al. The interactive walkway provides fit-for-purpose fall-risk biomarkers in the elderly: Comparison of zolpidem and suvorexant. Clin Transl Sci. 2024.
17. Murphy P, Kumar D, Zammit G, Rosenberg R, Moline M. Safety of lemborexant versus placebo and zolpidem: effects on auditory awakening threshold, postural stability, and cognitive performance in healthy older participants in the middle of the night and upon morning awakening. J Clin Sleep Med. 2020;16(5):765–773.
18. Inoue Y, Nishida M, Kubota N, Koebis M, Taninaga T, Muramoto K, et al. Comparison of the treatment effectiveness between lemborexant and zolpidem tartrate extended-release for insomnia disorder subtypes defined based on polysomnographic findings. J Clin Sleep Med. 2023;19(3):519–528.
19. Sattari N, Dave A, Niknazar H, Chen IY, Neikrug AB, Mander BA, et al. Effects of lemborexant and zolpidem on sleep electroencephalography in older adults with insomnia: a randomized trial. Sleep Adv. 2026;7(1):zpag017.
20. McElroy H, Desai C, Bate C, et al. A systematic review and network meta-analysis of treatments for insomnia in older adults. Sleep Med Rev. 2021;59:101512.
21. Bubu OM, Brannick M, Mortimer J, Udinwe U, John Y, Kaur I, et al. Sleep, cognitive impairment, and Alzheimer's disease: A

DOI: 10.52629/jamsa.v12i1.1119

Supplementary Files

Comparative Efficacy and Safety of Dual Orexin Receptor Antagonists (DORAs) Versus Z-Drugs for Insomnia in Older Adults : A Systematic Review

DOI: 10.52629/jamsa.v12i1.1119

Appendix 1. PICO Framework

Population	Intervention	Comparison	Comparison
Older adult patients (aged ≥50 years) formally diagnosed with primary insomnia or insomnia comorbid with other conditions.	Dual Orexin Receptor Antagonists (Lemborexant, Daridorexant and Suvorexant)	Traditional non-benzodiazepine hypnotics (Z-drugs), including agents such as Zolpidem, Zopiclone	• Primary: Incidence of adverse events (specifically focusing on falls, morning hangover/ somnolence, and next-morning cognitive/ psychomotor impairment). • Secondary: Sleep onset latency (SOL), wake after sleep onset (WASO), and overall sleep quality improvement

Appendix 2. Search Strategy

Database	Search Strategy	Search Results
PubMed	("Sleep Initiation and Maintenance Disorders"[Mesh] OR "insomnia"[tiab] OR "sleep disorder*"[tiab]) AND ("lemborexant"[tiab] OR "daridorexant"[tiab] OR "Dayvigo"[tiab] OR "Quviviq"[tiab]) AND ("zolpidem"[Mesh] OR "zolpidem"[tiab] OR "zopiclone"[tiab] OR "Ambien"[tiab] OR "Imovane"[tiab])	27
ScienceDirect	(insomnia OR "sleep disorder") AND (lemborexant OR daridorexant OR suvorexant) AND (zolpidem OR zopiclone)	53
Proquest	Box 1: insomnia OR "sleep disorder" OR "sleep disorders" Box 2 (AND): lemborexant OR daridorexant OR Dayvigo OR Quviviq Box 3 (AND): zolpidem OR zopiclone OR Ambien OR Imovane	140
Sage Journal	(insomnia OR "sleep disorder" OR "sleep disorders") AND (lemborexant OR daridorexant OR Dayvigo OR Quviviq) AND (zolpidem OR zopiclone OR Ambien OR Imovane)	15

DOI: 10.52629/jamsa.v12i1.1119

Appendix 3. PRISMA result

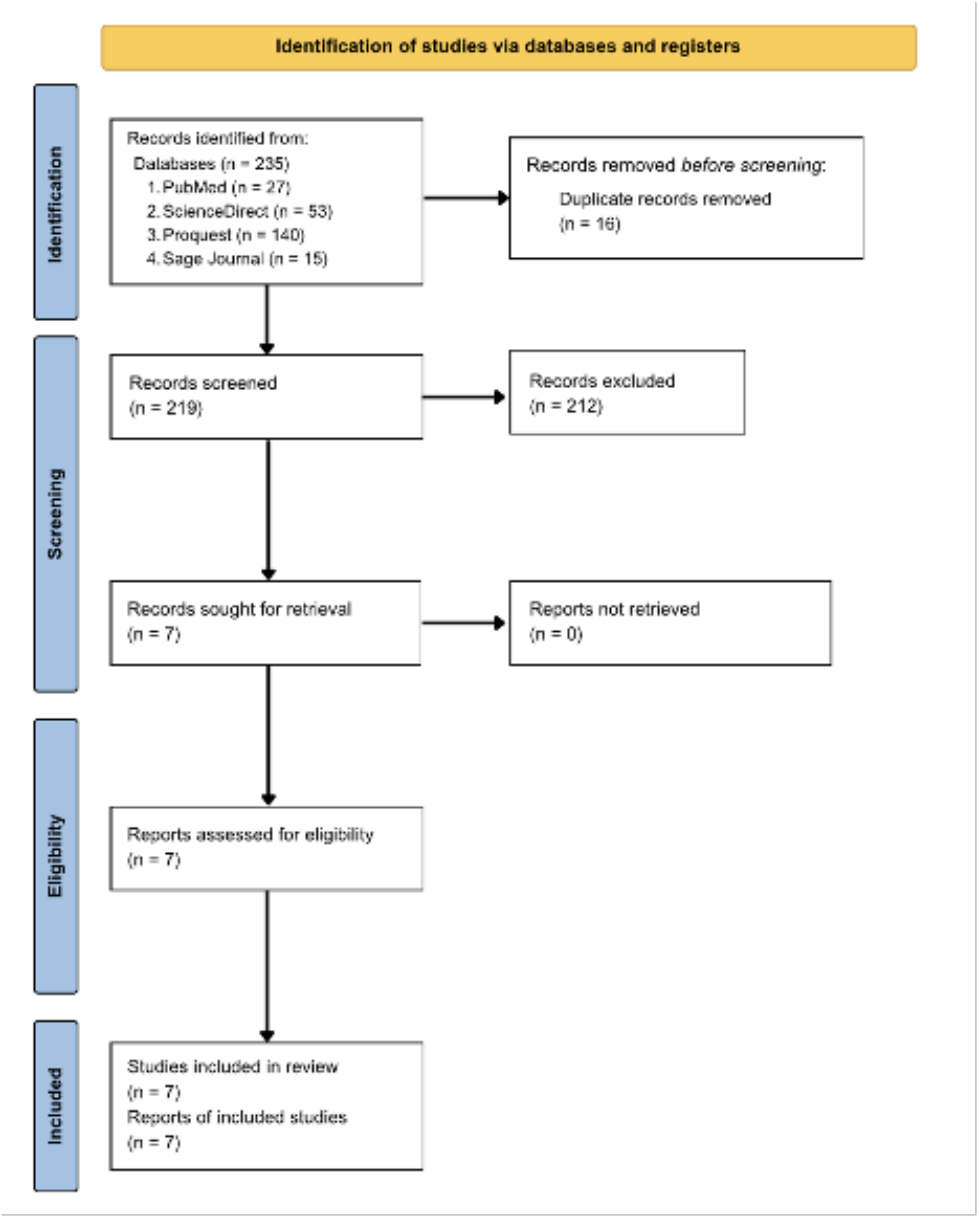

Figure 1: PRISMA Flowchart

DOI: 10.52629/jamsa.v12i1.1119

Appendix 4. Study Characterisation

First Author, year	Design	Population (N,Stage Age)	Intervention (I)	Comparator (C)	Protocol (Dose/ Freq)	Outcomes
Koopmans , 2024	RCT	N: 18, Stage: Healthy elderly, Age: 66 - 88 (mean: 71.9)	Suvorexant	Zolpidem and placebo	Dose: 10 mg suvorexant, 5 mg zolpidem, or placebo. Frequency: Single dose administered in the morning following a 2-hour fast	Interactive Walk Way (IWW) Body Sway Adaptive tracking (%) Timed Up and Go
Inoue , 2023	RCT	N: 1006 Stage: Participants met the DSM-5 criteria for insomnia disorder Age: ≥ 55 years	Lemborexant	Zolpidem and placebo	Single daily oral dose (5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo) for 30 nights, taken within 5 minutes of the time they intended to sleep	SOL WASO TST
Rosenberg , 2019	RCT	N: 1006 Stage: Participants met the DSM-5 criteria for insomnia disorder Age: 63.9 (Mean)	Lemborexant	Zolpidem and placebo	5 mg of lemborexant, 10 mg of lemborexant 6.25 mg of zolpidem, or placebo in a 5:5:5:4 ratio.	Latency to persistent sleep WASO Sleep efficiency (%) AEs
Murphy , 2020	CrossOver	Healthy Older Adults Women ≥ 55 y.o., Men ≥ 65 y.o. (Crossover design)	Lemborexant 5 mg and 10 mg (Single-dose, 4-period crossover)	Placebo Zolpidem ER (6.25 mg)	Dose: 5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo. Single dose at bedtime in a 4-period crossover design.	5 mg and 10 mg showed no significant impairment in MOTN postural stability or AAT compared to placebo.
Moline , 2021	SUNRISE-1 Cohort	Older Adults with Insomnia ≥ 55 y.o. (SUNRISE-1 Cohort)	Lemborexant 5 mg and 10 mg (1 month)	Placebo Zolpidem ER (6.25 mg)	Dose: 5 mg or 10 mg lemborexant, 6.25 mg zolpidem ER, or placebo. Single daily oral dose at bedtime for 1 month.	LEM significantly increased TST and REM sleep, and decreased latency to REM compared to both placebo and Zolpidem.

DOI: 10.52629/jamsa.v12i1.1119

First Author, year	Design	Population (N,Stage Age)	Intervention (I)	Comparator (C)	Protocol (Dose/ Freq)	Outcomes
Sachiko, 2022	CrossOver	Healthy Elderly Subjects Older adults (Evaluated via crossover)	Suvorexant (Low dose) Ramelteon	Placebo Zolpidem	Dose: Suvorexant (low dose), ramelteon, zolpidem, or placebo. Single dose at bedtime in a crossover design	Low-dose Suvorexant and Ramelteon demonstrated significantly fewer next-morning residual effects compared to Zolpidem.
Sattori , 2026	RCT	Older Adults with Insomnia ≥ 55 y.o. (RCT)	Lemborexant (Acute and chronic use)	Zolpidem	Dose: Lemborexant or zolpidem. Acute and chronic administration at bedtime	ZOL & LEM different effects LEM better maintains physiological sleep microarchitec-ture

DOI: 10.52629/jamsa.v12i1.1119

Appendix 5. Risk of Bias Assessment

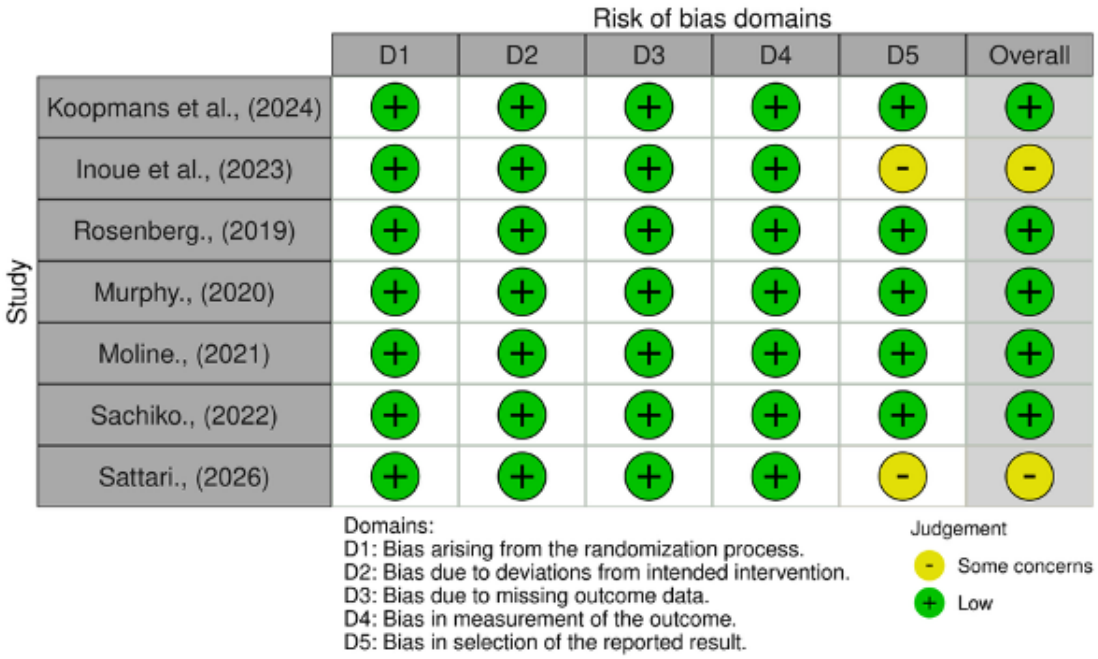

Figure 2: Risk of Bias Traffic Plot

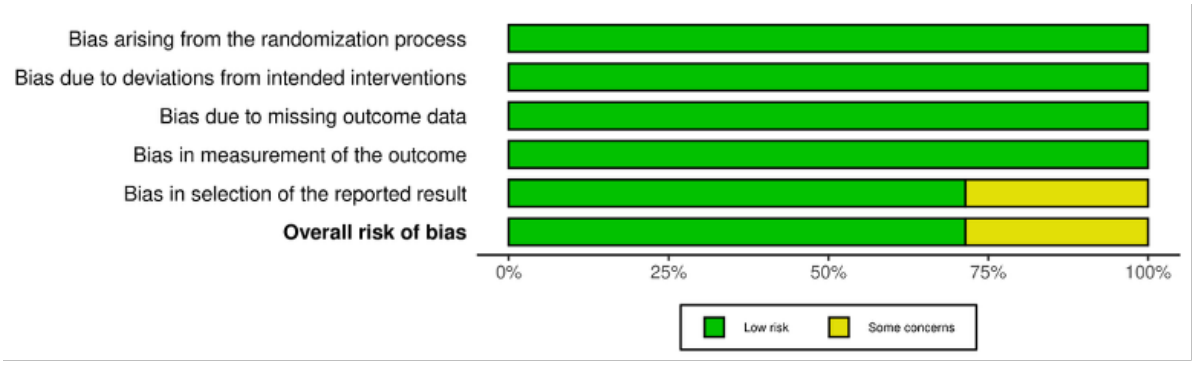

Figure 3: Risk of Bias Conclusion

DOI: 10.52629/jamsa.v12i1.1119

Appendix 6. Author's Contributions

Credit Role	Author(s)
Conceptualization	Jason Conrad Tanely
Methodology	Frederick Karel Himawan & Jason Conrad Tanely
Investigation / Literature Search	Frederick Karel Himawan & Jason Conrad Tanely
Data Curation / Screening	Jason Conrad Tanely , Frederick Karel Himawan , Given Anugraha Wijaya
Formal Analysis	Given Anugraha Wijaya
Writing – Original Draft	Jason Conrad Tanely , Frederick Karel Himawan , Given Anugraha Wijaya
Writing – Review & Editing	Jason Conrad Tanely , Frederick Karel Himawan , Given Anugraha Wijaya
Project Administration	Given Anugraha Wijaya

DOI: 10.52629/jamsa.v12i1.1105

Early Goal Directed Training vs Passive Therapy for Functional Independence of Infants with Cerebral Palsy: Systematic Review and Meta-Analysis

Tricia Arella Taslim[1], Dhana Carissa Wangjaya[1], Deyra Maheswari Wistara[1], Febry Liana[1], Fadhlurrahman Ahmad Ariq Bachtiar[1]

[1]Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

Background: Cerebral palsy (CP) is the leading cause of childhood motor disability, especially in LMICs. Early intervention is critical, yet the efficacy of goal-directed training compared to passive/standard therapies remains debated.

Objective: This study aims to evaluate the effectiveness of early goal-directed interventions on improving functional independence in infants and children with or at high risk of CP.

Methods: This systematic review and meta-analysis were conducted in accordance with PRISMA guidelines. A comprehensive literature search was performed to identify randomized controlled trials (RCTs) comparing goal-directed training with passive therapy or standard care. Methodological quality was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool. Data were synthesized using a random-effects model to calculate the Standardized Mean Difference (SMD).

Results: Five RCTs involving 302 participants were included. Meta-analysis revealed that goal-directed training showed a positive trend toward improved functional outcomes compared to passive therapy, although it did not reach statistical significance (SMD 0.19; 95% CI -0.09 to 0.47; p=0.1398). Heterogeneity across studies was remarkably low (I2 = 0), indicating a consistent intervention effect across diverse age groups and therapeutic modalities. Risk of bias assessment identified "some concerns" in three studies primarily due to missing outcome data, while two trials were rated as low risk.

Conclusion: Goal-directed training demonstrates a promising and consistent clinical trend in enhancing functional independence for children with CP. Despite the current lack of statistical significance, the negligible heterogeneity supports the integration of active, goal-focused exercises as a potentially effective and stable strategy for early neurorehabilitation.

Keywords: Cerebral palsy, early intervention, goal-directed training, functional independence, meta-analysis

Category: Review Articles

Date Received: 16 May 2026

Date Accepted: 22 June 2026

Correspondence to:

Tricia Arella Taslim

Atma Jaya Catholic University of Indonesia

taslimtricia@gmail.com

DOI: 10.52629/jamsa.v12i1.1105

Introduction

Cerebral palsy (CP) is the most common permanent motor disability in children worldwide. The prevalence of CP ranges from 1.6 cases per 1,000 people in high-income countries to 4 cases per 1,000 people in lower-middle-income countries (LMICs). Many children with CP still do not receive adequate intervention or therapy.[1] The global impact of this condition has far exceeded expectations. According to data from the World Health Organization (WHO) and the 2019 Global Burden of Disease Study, approximately 8 million children in 204 countries live with Cerebral Palsy (CP). The majority of these children, 96%, are concentrated in LMICs.[1] This condition shows that CP is not only a clinical problem for each individual country, but rather an urgent global problem that needs to be addressed, especially in developing countries.

The effects of Cerebral Palsy (CP) are not limited to motor skills but encompass the entire spectrum of a child's life. According to the International Classification of Functioning, Disability and Health (ICF) framework, this condition disrupts bodily functions and limits a child's involvement in daily activities, education, and social interactions. Collectively, these barriers reduce the quality of life for both the child and their family.[2] Furthermore, there is significant evidence that increasing the autonomy of children with CP in daily activities contributes to reducing the psychological burden on caregivers. This directly impacts the overall quality of life of the family.[3] Thus, functional independence is a central clinical outcome that should be the primary goal in any rehabilitation program for children with CP.

The strongest scientific basis for early intervention in CP is neuroplasticity. Evidence shows that the developing brain is highly responsive to the quality, quantity, and timing of early sensorimotor experiences, and the infant's active participation is an indispensable element in promoting this neuroplasticity.[4] Most lesions underlying CP occur in the second half of gestation, when brain development is at its peak the variations in the timing of injury result not only in different lesions but also in different neuroplastic responses.[5] Interventions initiated early, during the critical period of brain plasticity and before musculoskeletal and central nervous system maladaptations occur, are believed to offer the best opportunity to optimize the developmental trajectory of children with CP.[6]

For decades, the dominant rehabilitation approach used in infants and children with CP has been passive therapy, particularly Bobath-based Neurodevelopmental Therapy (NDT), which focuses on normalizing muscle tone and providing external movement facilitation by a therapist. However, the effectiveness of this approach has been increasingly questioned. A meta-analysis published in the journal Pediatrics (2022) concluded that activity-based interventions were more effective than NDT in improving motor function, NDT was no more effective than a control group, and increasing the dose of NDT did not result in greater improvement; a strong recommendation was made to discontinue the use of NDT in the management of CP.[7] Similarly, global CP experts have unequivocally stated that the available evidence indicates Bobath/NDT is ineffective and should therefore be de-implemented in clinical practice.[8] Such passive approaches also align with the principles of neuroplasticity, as evidence suggests that excessive use of external facilitation or physical guidance during children's movement is not recommended, and treatment techniques aimed at inhibiting muscle tone have not been shown to be effective.[9]

In response to these limitations, the rehabilitation paradigm has shifted to active, goal-directed training (GDT) approaches. Current literature positions GDT as a method for improving function in children with CP, in which the child actively practices a desired goal or task; this approach uses varied and repetitive activities to promote neuroplasticity and functional improvement and is now considered a standard of care for CP.[9] One GDT program specifically designed for infants is GAME (Goals–Activity–Motor Enrichment), which combines intensive goal-based motor training, parent education, and environmental enrichment. Unlike NDT, this program focuses on task feasibility and environmental context, with the therapist acting as a "change agent" facilitating the infant's exploration of motor solutions.[10] The LEAP-CP RCT, published in Pediatrics (2024), in infants with CP in a low-income country, found that although there was no overall effect size, a modified intention-to-treat analysis showed that the goal-directed intervention resulted in significant improvements in motor skills in ambulatory children with CP, consistent with what is known about targeted goal-based training.[11]

However, several critical gaps in scientific evidence remain unaddressed. To date, data on the efficacy of early intervention in infants with CP remains very limited, as the diagnosis of CP is typically not made until between 12 and 24 months of age, often missing the most critical intervention window.[12] More importantly, despite the proven effectiveness of GDT, variability in

DOI: 10.52629/jamsa.v12i1.1105

its implementation has contributed to the gap between research evidence and daily clinical practice.[13] Research directly and head-to-head comparing early goal-directed training with passive therapy in infants with CP particularly with functional independence as the primary focus remains very limited, particularly in LMIC contexts. Recent guidelines from China (2024) also emphasize that structured, goal-based rehabilitation is the backbone of CP management, but its implementation remains uneven.[14]

This gap is the primary justification for this research. The current situation reflects a reality where passive therapy is still widely practiced in various rehabilitation facilities, while scientific evidence consistently and increasingly demonstrates that active, goal-based approaches from an early age are superior in maximizing neuroplasticity and increasing functional independence. This study aims to generate stronger comparative evidence regarding the superiority of early goal-directed training over passive therapy for functional independence in infants with CP, thus providing a basis for updating clinical guidelines and pediatric rehabilitation practices globally.

Method

Study Design and Protocol Registration

This study was designed as a systematic review and meta-analysis to compare the effectiveness of early goal-directed training with passive therapy for functional independence in infants with cerebral palsy. This review integrates evidence from randomized controlled trials to provide a comprehensive summary of the existing data. Reporting of the study results follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency, completeness, and reproducibility of the methods.[15]

The review protocol was established in advance to guide the methodological approach of this study. It was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number (CRD420261394788). The protocol was registered to ensure transparency throughout the review process and to limit the possibility of reporting bias. Any modifications to the original protocol were clearly stated and justified in the final report.[16]

Eligibility Criteria

Studies were selected according to the Population, Intervention, Comparison, Outcome, and Study Design (PICOS) framework. Eligible studies were randomized controlled trials involving infants and children diagnosed with cerebral palsy or identified as being at high risk of developing cerebral palsy. The intervention group consisted of early goal-directed or activity-based rehabilitation programs aimed at improving motor function and functional independence. These interventions included structured active therapies such as Goals–Activity–Motor Enrichment (GAME), Baby-CIMT, Baby-BIM, LEAP-CP, and other task-oriented training approaches.

Comparator groups included passive therapy, conventional neurodevelopmental treatment, standard rehabilitation, or other non-goal-directed therapeutic approaches. The primary outcomes assessed were functional independence, activity performance, participation, and motor function measured using validated assessment instruments, including the Gross Motor Function Measure (GMFM), Pediatric Evaluation of Disability Inventory (PEDI/PEDI-CAT), Hand Assessment for Infants (HAI), Canadian Occupational Performance Measure (COPM), or comparable standardized functional outcome tools.

Only full-text articles published in English between January 2016 and May 2026 were included. Conference abstracts, review articles, case reports, editorials, study protocols, animal studies, and non-comparative studies were excluded. Studies with insufficient quantitative data for effect size calculation were also excluded from the meta-analysis.

Information Sources and Search Strategy

Relevant studies were identified through searches of multiple electronic databases, including PubMed, ScienceDirect, ProQuest, Wiley Online Library, and MedRxiv. MedRxiv was included to capture gray literature, identify emerging evidence, and minimize potential publication bias. To systematically address the lack of peer review in preprint studies, a sensitivity analysis using a leave-one-out approach was planned a priori to evaluate whether the inclusion of these preprint data significantly or systematically distorted the primary pooled meta-analysis findings. Studies published from January 2016 until the final search date (7 May 2026) were considered for inclusion. To ensure comprehensiveness, the reference lists of all included studies and relevant review articles were manually screened, and a snowballing approach was applied to identify additional eligible studies.

DOI: 10.52629/jamsa.v12i1.1105

The search strategy was developed using database-specific search strings that incorporated controlled vocabulary (where applicable), free-text terms, and Boolean operators ("AND" and "OR"). Search terms were related to cerebral palsy, high-risk infants, goal-directed interventions, and functional independence outcomes. Search strategies were adapted according to the indexing structure and search requirements of each database. The complete database-specific search strategies, including all search terms, Boolean operators, and MeSH headings, are presented in Supplementary Appendix 1.

Study Selection and Data Collection

Two reviewers independently screened titles and abstracts of all retrieved records according to the predefined eligibility criteria. Potentially eligible studies subsequently underwent full-text assessment by the same reviewers. Any disagreements regarding study selection were resolved through discussion and consensus.

Data extraction was performed independently by two reviewers using a standardized extraction form. Extracted data included study characteristics, participant demographics, intervention details, outcome measures, and study outcomes. Any discrepancies were resolved through discussion and review of the original articles until consensus was reached. Risk of bias assessment was conducted independently by two reviewers using the Cochrane Risk of Bias 2 (RoB 2) tool. Disagreements were resolved through discussion and consensus.

Risk of Bias Assessment

The RCT study was assessed using the Cochrane Risk of Bias tool version 2 (RoB 2.0) and visualized using the ROBVIS visualization.[17,18] The assessment covered five key domains, including the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The results of the assessment were explicitly used to interpret the strength of the clinical evidence and were visualized using specialized tools to maintain transparency in the quality of the included studies.

Statistical Analysis

A quantitative synthesis was performed using meta-analysis to evaluate the effectiveness of early goal-directed and activity-based interventions on functional independence and motor outcomes in infants and children with or at high risk of cerebral palsy. All statistical analyses and quantitative poolings were conducted using Review Manager (RevMan) software, version 5.4 (The Cochrane Collaboration, 2020). To evaluate the overall impact of the intervention on functional capacity, data from diverse outcome measures (including GMFM for gross motor function, HAI for upper limb function, and PEDI-CAT or COPM for comprehensive functional domains) were pooled using the Standardized Mean Difference (SMD) with corresponding 95% confidence intervals (CI). The use of SMD is justified as it standardizes heterogeneous scales into a uniform metric, allowing for an aggregate estimation of global functional improvement. To minimize conceptual bias, data were hierarchically selected based on the primary domain targeted by the intervention when a study reported multiple scales.

A random-effects model was applied to account for anticipated clinical and methodological heterogeneity among studies, including differences in intervention protocols, treatment duration, participant characteristics, and outcome measurement tools. Statistical heterogeneity was assessed using the I^2 statistic, with values above 50% considered indicative of substantial heterogeneity. Statistical significance was defined as a two-tailed p-value < 0.05.

As an exploratory measure, publication bias was evaluated qualitatively through visual inspection of funnel plot asymmetry. Although visual inspection of the plot may demonstrate relative symmetry, this interpretation must be treated with extreme caution. Standard methodological guidelines indicate that publication bias assessments using funnel plots are unreliable and lack statistical power when a meta-analysis includes fewer than 10 studies. Therefore, the presence of small-study effects or selective publication cannot be definitively ruled out based on our limited sample size. Sensitivity analysis was conducted using a leave-one-out approach to assess the robustness and stability of the pooled effect estimates.

Result

Study Selection

A systematic literature search was conducted across five electronic databases, including PubMed, ScienceDirect, Wiley Online Library, ProQuest, and medRxiv. A total of 314 records were identified through database searching, consisting of 191 records from ProQuest, 99 from ScienceDirect, 12 from Wiley, 10 from medRxiv,

DOI: 10.52629/jamsa.v12i1.1105

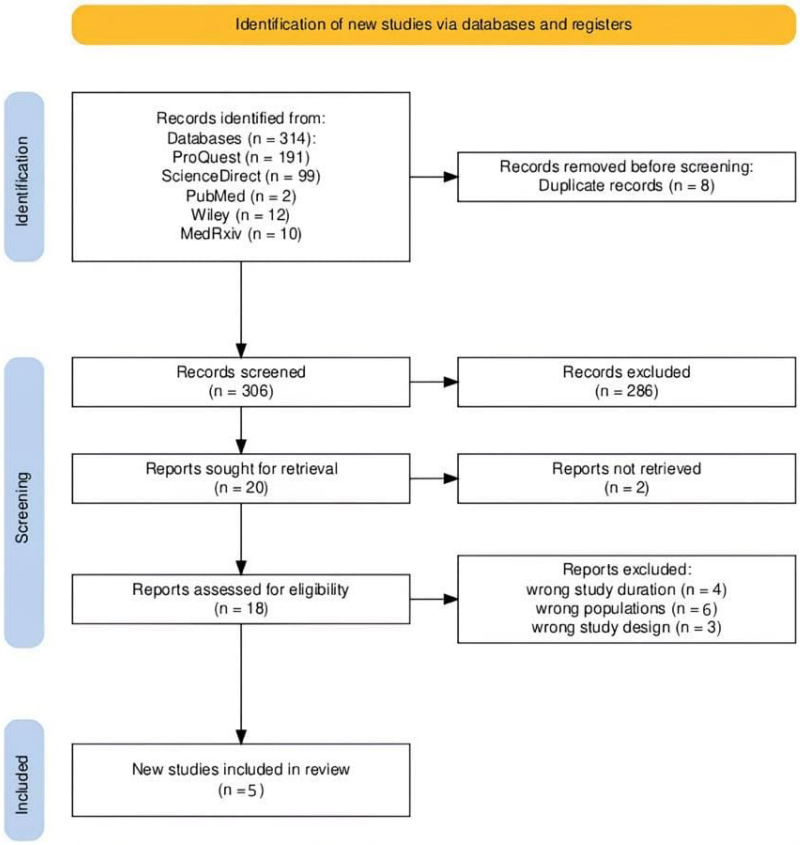

Figure 1: PRISMA Flowchart

and 2 from PubMed.

After removal of 8 duplicate records, 306 studies underwent title and abstract screening. Of these, 286 records were excluded due to irrelevance to the study objectives. Subsequently, 20 full-text articles were sought for retrieval, of which 18 articles were successfully assessed for eligibility, while 2 reports could not be retrieved.

During full-text assessment, 12 studies were excluded for the following reasons: inappropriate study duration (n = 4), irrelevant population (n = 6), and unsuitable study design (n = 3). Ultimately, 5 studies met the eligibility criteria and were included in the systematic review and meta-analysis. The study selection process is summarized in the PRISMA flow diagram (Figure 1).

Study Characteristics

The baseline and methodological characteristics of the five included trials are systematically summarized in Table 1.

Risk of Bias Analysis

The methodological quality and risk of bias included randomized controlled trials were independently assessed by the Cochrane Risk of Bias 2 (RoB 2) tool.[19] Each study was evaluated across five distinct domains: (1) bias arising from the randomization process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in measurement of the outcome; and (5) bias in selection of the reported result. The assessment of the risk of bias for the five included studies was conducted using the Cochrane Risk of Bias 2 (RoB 2) tool, as illustrated in Figures 2 and 3.

Overall, the studies demonstrated a high level of methodological quality, with two trials (Boyd et al., 2025 and Armstrong et al., 2020) achieving a "low risk of bias" across all evaluated domains. These studies implemented robust randomization and allocation concealment procedures, alongside effective blinding of outcome assessors.

In contrast, three studies (Morgan et al., 2016; Eliasson et al., 2018; Benfer et al., 2024) were identified as having

DOI: 10.52629/jamsa.v12i1.1105

Table 3. Characteristics of Included Trials

Author, Year	Study Location	Sample (n)	Study Design	Age	Diagnosis/ Risk	Type of Cerebral Palsy	Dose (Frequence or Duration of Intervention)	Setting	Intervention	Comparison	Primary Outcome (measurement tool)	Secondary Outcome
Benfer, et al. (2024)	India (West Bengal, LMIC)	n = 153 (LEAP-CP = 77, HA = 76)	RCT (single-blind, multisite)	12–40 weeks corrected age (CA). Mean 7.1 ± 2.7 months CA	High Risk of CP	Not Classified	15 fortnightly home visits (≈7.5 months) until 18 months CA Peer-delivered; dose-matched between arms	Home-based	LEAP-CP (goal-directed, parent-delivered, multidomain)	Health Advice (WHO Key Family Practices)	PEDI-CAT Mobility domain + DASS (caregiver mental health)	PDMS-2, BSID-III, COPM, HINE, PSOC, Near Detection Scale, HOME Inventory
Morgan, et al. (2016)	Australia (Sydney)	n = 30 (GAME = 15, SC = 15)	RCT (single-blind, parallel group)	3–6 months CA at enrolment, Mean enrolment: 15.7–20.1 weeks CA	High Risk of CP	Not Classified	16 weeks, weekly home visits by therapist	Home-based + hospital visit for assessments	GAME (Goals–Activity–Motor Enrichment; motor learning + enrichment)	Standard Care (routine community therapy)	PDMS-2 (Peabody Developmental Motor Scales-2)	BSID-III, GMFM-66, COPM
Eliason, et al. (2018)	Sweden (Stockholm)	n = 37 (Baby CMIT: 18, Massage: 13)	Exploratory RCT (evaluator linded, parallel group)	3–8 months CA at entry Baby-CIMT: mean 6.1 ± 1.7 months Massage: mean 5.0 ± 1.6 months	High Risk of CP	Unilateral Cerebral Palsy	2 × 6-week training periods separated by 6-week pause (18 weeks total)	Home-based	Baby-CIMT (non-affected hand restrained; parent-led, OT-coached)	Baby-Massage (full-body massage, 1×/day, parent-led)	HAI Affected Hand Score	AHA, PSCS, Feasibility Questionnaire
Boyd, et al. (2025)	Australia and US (not specified)	n = 144 (72 for each group) --> 96/144 joined because recruitmentnya prematurely because of the COVID-19 pandemic.	Single-blinded RCT	3-9 months of corrected age	High Risk of CP	Unilateral Cerebral Palsy	1-3 sessions/day, 5 days/week for 6-9 months, according to age at study entry. Bimonthly home visits by pediatric therapist	Home-based	Baby-CMIT	Baby-BIM	HAI Affected Hand Score	Mini-AHA, AHA, BSID-III, PEDI-CAT, EA-SR, DASS-21,
Armstrong, et al. (2020)	Brisbane, Australia	n = 21 (11 with intervention, 11 usual care)	Parallel grouped RCT	6-18 years old	CP (GMFCS level II until IV)	Not Classified	1 hour sessions at a tertiary children's hospital and 1 hour home exercise programme (HEP) for 8 weeks. Included 30 minutes of goal-directed training programme from Canadian Occupational Performance Measure (COPM) and 30 minutes of FES cycling	home and hospital based??	Goal-directed training and FES cycling	Usual care (physiotherapy, speech therapy, occupational therapy)	GMFM (Gross Motor Function Measure) and COPM (Canadian Occupational Performance Measure)	Peak cycling powe and resistance, Peadiatric Evaluation of Disability Inventory Computer Adaptive Test (PEDI-CAT), and Participation and Environment Measure – Child and Youth (PEMCY)

DOI: 10.52629/jamsa.v12i1.1105

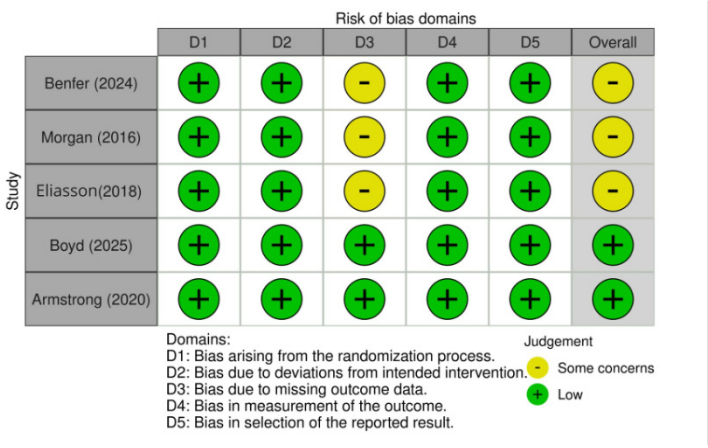

Figure 2: Risk of Bias domains of RCT studies

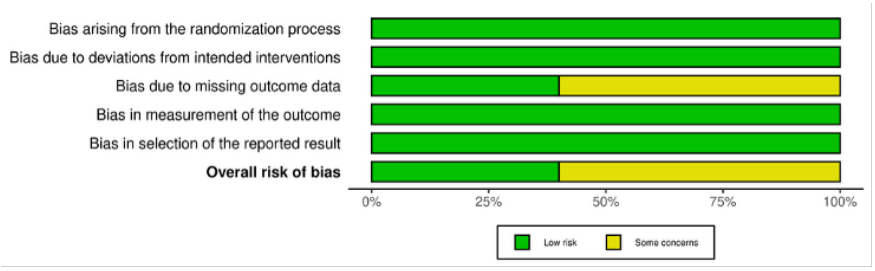

Figure 3: Overall risk of bias of RCT studies

"some concerns" in their overall judgment. In Benfer et al. (2024) and Eliasson et al. (2018), these concerns were primarily related to Domain 3 (bias due to missing outcome data) and Domain 2 (deviations from intended interventions), often due to participants being excluded after randomization or high attrition rates. Similarly, Morgan et al. (2016) was noted for "some concerns" in Domain 3 due to the loss of follow-up data in the intervention group. Despite these specific issues, none of the included studies were rated as "high risk," and the measurement of primary outcomes remained consistently at low risk of bias across the evidence base.

Quantitative Synthesis

Functional Motor Outcomes

A random-effects meta-analysis using standardized mean difference (SMD) was performed due to differences in outcome scales across studies. The pooled analysis demonstrated a small positive effect favoring early goal-directed and activity-based interventions compared with passive therapy or standard care (SMD = 0.19; 95% CI: -0.09 to 0.47). However, the overall effect was not statistically significant because the confidence interval crossed the null value (0). Heterogeneity among studies was low (I^2 = 0%), indicating consistent findings across included studies. These findings suggest that activity-based and goal-directed interventions demonstrate promising trends toward improving modest improvements in functional motor outcomes compared with passive therapy, although current evidence remains insufficient to demonstrate definitive effectiveness.

Forest Plot Interpretation

The forest plot demonstrated that most included studies favored the intervention group, as indicated by positive SMD values. According to Cohen's convention, the pooled estimate (SMD = 0.19; 95% CI: -0.09 to 0.47) represents a small effect size magnitude. The studies by Morgan et al. (2016) and Eliasson et al. (2018) showed the largest positive effects.[20,21] Visual inspection of the plot reveals a substantial overlap among the confidence intervals of individual trials, indicating statistical consistency. However, several studies exhibited wide confidence intervals crossing the line of no effect, reflecting limited statistical precision and uncertainty driven by the small cumulative sample size. The pooled diamond slightly favored the intervention group but crossed the null line, indicating non-significant overall findings. The study weights ranged from 7.3% to 33.4%, with Boyd et al. (2024) and Benfer et al. (2024)

DOI: 10.52629/jamsa.v12i1.1105

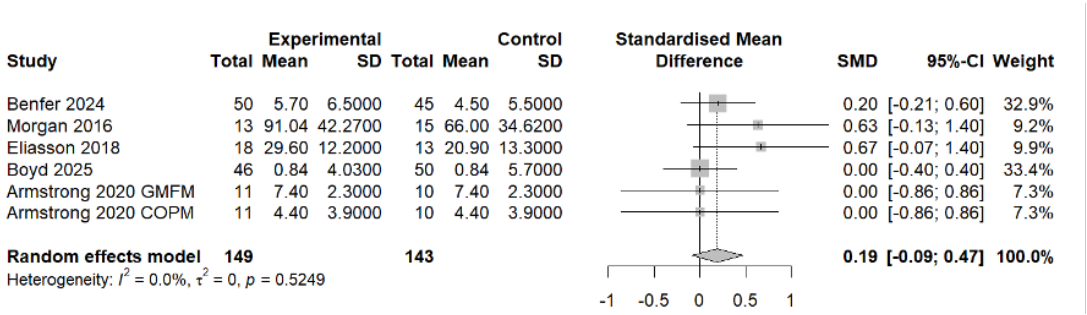

Figure 4: Forest Plot of Functional Motor Outcomes

CI: Confidence Interval; SMD: Standardized Mean Difference; SE: Standard Error.

contributing the greatest influence to the pooled analysis due to larger sample sizes.[22, 23]

Publication Bias

Visual inspection of the funnel plot did not demonstrate marked asymmetry. However, interpretation should be approached cautiously because the number of included studies was small (<10 studies), limiting the reliability of publication bias assessment.

Sensitivity Analysis

Leave-one-out sensitivity analysis demonstrated stable pooled estimates across all iterations. Exclusion of individual studies did not substantially alter the overall effect size, suggesting that no single study disproportionately influenced the meta-analysis results. The pooled effect sizes after sequential omission of studies ranged from SMD 0.14 to 0.28, with all analyses remaining statistically non-significant. Heterogeneity remained consistently low (I^2 = 0%). The exact step-by-step omitted-study analysis, detailing the shifting SMD values and specific confidence intervals for each iteration, has been comprehensively tabulated and provided in Supplementary Table S1.

Overall Interpretation

Overall, early goal-directed and activity-based interventions demonstrated a trend toward improved functional motor outcomes in infants with cerebral palsy or high risk of cerebral palsy compared with passive therapy or standard care. Nevertheless, the pooled effect did not reach statistical significance.

Despite this, the consistently positive direction of effect and low heterogeneity \suggest potential clinical benefits of active, goal-oriented early intervention approaches. Additional high-quality randomized controlled trials with larger sample sizes and standardized outcome measures are needed to clarify the magnitude of benefit.

Discussion

The findings of this meta-analysis reveal a consistent and positive but non-significant trend toward the

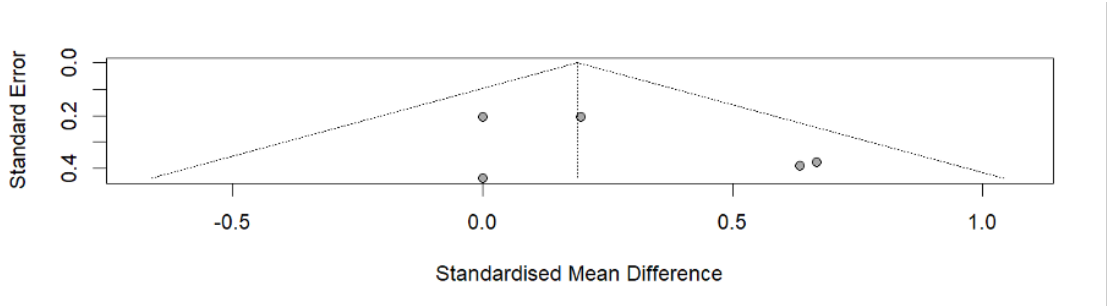

Figure 5: Funnel Plot for Publication Bias Assessment

Horizontal axis represents Standardized Mean Difference (SMD); vertical axis represents Standard Error (SE) to assess small-study effects.

DOI: 10.52629/jamsa.v12i1.1105

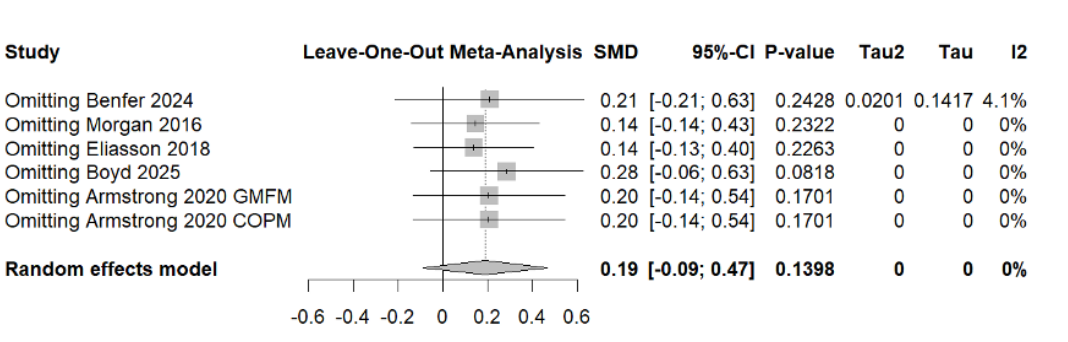

Figure 6: Sensitivity Analysis Chart

Omitted-study analysis applying a leave-one-out framework to assess the robustness of the pooled estimate.

efficacy of early active interventions for infants at high risk of cerebral palsy. Although the pooled analysis falls short of demonstrating definitive clinical superiority, several studies by Armstrong et al. (2020), Benfer et al. (2024), Choudhary et al. (2013), Boyd et al. (2025), Eliasson et al. (2018), and Morgan et al. (2016) Reported an undeniable increase in motor function, such as the upper limb movements and cognitive outcomes.[20-22,24,25] However, this therapeutic benefit may vary depending on the child's baseline functional severity, therefore an adjusted interpretation of result according to this study is mandatory. Benfer et al. (2024) reported that LEAD-CP appeared to provide greater benefits than HA, particularly in ambulant children with cerebral palsy.[23]

Application of Early Active Goal-based Intervention in Pathophysiology of Cerebral Palsy (CP)

The superiority of early active, goal-directed training (GDT) over passive modalities like Neurodevelopmental Treatment (NDT)/Bobath lies in its alignment with activity-dependent neuroplasticity. Cerebral palsy (CP) arises from a non-progressive injury to the developing brain during peak developmental activity, directly disrupting the normal activity-dependent synaptic competition of the corticospinal tract (CST). Variations in the timing of injury result not only in different lesions but also in different neuroplastic responses.[26] While passive therapies fail to trigger neural reorganization because they lack deliberate CST activation from the infant, active GDT explicitly exploits motor learning mechanisms. By motivating self-initiated, repetitive movements, GDT repeatedly activates remaining CST axons, thereby strengthening weak synaptic connections, inducing white and gray matter changes such as increased myelination, and preventing the pathological cycle of learned non-use.[26]

The most relevant neuroplasticity mechanism in the context of CP rehabilitation is activity-dependent plasticity, the nervous system's capacity to reorganize itself structurally and functionally in response to active, repetitive motor experiences. The basic principles of experience-dependent neural plasticity have been comprehensively formulated by Kleim & Jones, encompassing specificity, repetition, intensity, and functional relevance. Understanding these principles is highly relevant for optimizing rehabilitation strategies after brain injury.[27] Motor interventions during the early years of life are considered critical for optimizing this activity-dependent neuroplasticity, but it is important to emphasize that activity-dependent reorganization can be both adaptive and maladaptive, so it is also the child's active role in the process.[9] This fundamentally differentiates active, goal-based therapy from passive therapy. In passive therapy approaches such as NDT/Bobath, the therapist physically guides or facilitates movement while the infant plays a passive role. This kind of approach fails to trigger activity-dependent plasticity because it does not produce a deliberate CST activation pattern from the child himself, and has been shown to be ineffective in harnessing neural plasticity.[28]

Neuroplasticity and the Response to Goal-Directed Training

The strongest pathophysiological argument for early intervention rests on the concept of the critical period of neuroplasticity, a specific window during early development where brain plasticity peaks and sensory experiences are necessary to build optimal cortical representations.[29] Outside this period, plasticity capacity is significantly reduced, and the same intervention produces a much weaker response. Data from gross motor function development curves indicate that the gross motor potential of most children

with CP plateaus before age 5, depending on GMFCS level. For example, three-quarters of children with GMFCS levels I and II achieve 90% GMFM scores by age 5, while children with GMFCS level IV plateau at around 30% by the same age.[30] Plasticity in the motor system has been shown to be more robust in the early years than in later years, and maladaptive changes that occur after CNS injury are difficult to reverse once established.[30] Neuroimaging studies confirm that children with CP possess cortical plasticity that can be beneficial.[32] Delayed intervention, such as waiting until a definitive diagnosis at 12–24 months of age, pathophysiologically misses part of this critical window of greatest responsiveness.

Early goal-directed training (GDT) is explicitly designed to take advantage ofmotor learning mechanisms that optimally utilize neuroplasticity. The developing brain is highly responsive to the quality, quantity, and timing of early sensorimotor experiences, and the infant's active participation is essential to foster this process.[31] Evidence from experimental models suggests that early intensive rehabilitation reverses locomotor impairments, reduces brain inflammation, and induces neuroplasticity. Structural MRI confirms that activity-based and goal-directed interventions induce changes in gray and white matter properties in the brain, including increased myelination.[32] Conversely, without encouragement to actively move, infants with cerebral palsy are at risk of a learned non-use phenomenon in which infants learn not to use their weaker limbs due to the lack of positive reinforcement, which becomes increasingly difficult to correct with age. This condition can initiate a pathological cycle that deteriorates structure and function and produces maladaptive plasticity. Therefore, it is critical to initiate active interventions early to prevent permanent musculoskeletal and central nervous system maladaptations.[33]

Different Assessments through Different Measurement Tools

Motor function in children with cerebral palsy can be assessed using different types of interventions and outcome measures. Because each assessment tool examines different areas of function, the outcomes reported across studies may vary. This suggests that no single assessment can be regarded as the best overall tool, as each instrument has its own purpose and evaluates specific aspects of a child's abilities. Importantly, motor outcomes and functional independence should be interpreted as distinct but complementary constructs. In this review, motor outcomes primarily refer to improvements in gross motor abilities and upper-limb function, whereas functional independence reflects to broader domains, including participation and independence in activities of daily living.

Boyd et al. (2024) found that Baby-CIMT did not show greater effectiveness compared with Baby-BIM, as both interventions produced similar improvements in hand function and upper limb development.[22] These findings indicate that different intervention approaches may still lead to comparable outcomes in children with cerebral palsy. In addition, Armstrong et al. (2020) reported that GMFM was not necessarily superior to PEDI-CAT because both assessments measure different dimensions of motor function.[24] GMFM is mainly used to evaluate gross motor abilities, including movement-related activities such as cycling. Meanwhile, PEDI-CAT places greater emphasis on the child's ability to carry out everyday activities independently. Therefore, variations in study outcomes may be influenced not only by the intervention itself but also by the specific focus of the assessment tool used in each study.

Cerebral palsy affects not only motor function but also sensory function. A study conducted by Chorna et al. (2015) combined 6 hours of daily modified Constraint-Induced Movement Therapy (CIMT) with high-intensity tactile enrichment. Specifically, the intervention utilizes sensory-motor kits where infants search for objects in textured media and "sticky mittens" to facilitate successful reaching, providing the consistent, non-noxious afferent feedback required to rewire damaged thalamocortical pathways. To validate the impact of these therapies, the study employed several assessment tools, such as the use of high-density event-related potentials (ERP) and kinematic motion analysis. These measures quantify changes in cortical sensory processing and movement smoothness that traditional behavioral scales might overlook. By aligning parent-driven sensory exercises with objective cortical tracking, this approach suggests that the remediation of "developmental disregard" is best achieved when clinical therapy is paired withassessments capable of tracking the brain's evolving ability to register and utilize tactile information for motor control.[34]

Strengths and Limitations

A key strength of this systematic review and meta-analysis is its strict focus on the critical "window

DOI: 10.52629/jamsa.v12i1.1105

of opportunity" in early development, specifically targeting children. By evaluating goal-directed, activity-based interventions against traditional passive handling, this study provides a highly specialized synthesis of how active motor learning drives functional independence, measured through variations of standardized tools. Furthermore, the relationship between pathophysiology, neuroplasticity, and the response to early goal-directed training is clearly described. However, several limitations must be acknowledged. The overall number of pure randomized controlled trials in this specific age bracket remains relatively small, resulting in an underpowered pooled sample size that may limit the statistical power to detect significant differences. Moreover, there is a notable clinical heterogeneity across the literature; this is reflected in the high variability of intervention protocols—specifically the variations in total dosage, frequency, and duration—alongside the inclusion of mixed-severity populations and differing outcome measures. Finally, the short follow-up duration across the included studies limits our ability to determine whether the functional gains achieved in infancy are sustained into later childhood.

Clinical Implication and Future Direction

The findings of this study support a significant trend shift in pediatric rehabilitation, moving away from traditional, passive neuromotor handling, such as passive stretching or isolated NDT without functional goals, toward active, task-specific, and goal-directed interventions. For clinicians and physical therapists, the evidence underscores that motor learning in infants is experience-dependent. Therefore, therapeutic sessions should be structured around self-initiated movements where the infant must actively solve a motor problem to achieve a rewarding outcome. Successful protocols like GAME and Small Steps heavily rely on environmental enrichment and parental coaching, so clinical practiceinto a more family-centered model is highly encouraged. Clinicians should prioritize training parents as active co-therapists who can seamlessly integrate goal-directed activities into the infant's daily home routine, thereby maximizing therapeutic dosage and enhancing the infant's real-world functional independence. [35,36]

To address the clinical heterogeneity observed in current literature, future studies should directly compare holistic, whole-body active interventions against targeted approaches, such as Baby-CIMT, to determine which protocols yield the highest efficacy for specific subgroups of cerebral palsy. Additionally, researchers should incorporate long-term longitudinal follow-ups to track the trajectories of functional independence and neuroplastic adaptation as these infants transition into school age. Furthermore, given the heavy reliance on home-based therapy, future studies should explore secondary outcomes more thoroughly, particularly looking into the cost-effectiveness of these interventions and their psychological impact on parental stress and quality of life

Conclusion

In conclusion, early goal-directed and activity-based interventions demonstrate a promising and consistent trend toward improving functional independence and motor outcomes in infants and children with or at high risk of cerebral palsy. Although the pooled meta-analysis did not reach statistical significance, the overall positive direction of effect, negligible heterogeneity, and robust sensitivity analysis support the potential clinical value of active, goal-oriented rehabilitation compared with passive therapy approaches. These findings reinforce the neuroplasticity-based rationale that early, repetitive, and self-initiated motor experiences are critical during the developmental window of maximal brain plasticity. Furthermore, the evidence highlights the importance of family-centered and environmentally enriched interventions, such as GAME and Baby-CIMT, in optimizing functional outcomes. Nevertheless, the limited number of high-quality randomized controlled trials, variations in intervention protocols, and lack of long-term follow-up data indicate that further large-scale and standardized studies are still required to establish definitive clinical recommendations and determine the long-term sustainability of these therapeutic benefits.

Acknowledgement

All authors contributed equally to this review. The tasks were fairly distributed, including literature screening, data analysis, and manuscript drafting. Each author was responsible for specific parts of the writing and revision process. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper. There was no funding or commercial sponsorship involved in the preparation of this systematic review.

References

1. Olusanya BO, Nair MKC, Boo NY, Davis AC, Hadders-Algra M, Wright SM. Cerebral palsy in young children: bridging the global data gap. The Lancet Global Health. 2025 Aug 11
2. Africa LE, Human A, Tshabalala MD. Participation patterns of children with cerebral palsy: A caregiver's perspective. African Journal of Disability. 2023 Jan 31;12.
3. Faeze Alvandi, Amini M, Narges Ghafarzadeh Namazi. Factors Affecting the Independence Level of 4-6-Year-Old Children with Cerebral Palsy in Activities of Daily Living. PubMed [Internet]. 2023 Jan 1;17(4):93–104. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10704291/
4. Damiano DL. The E-words for promoting development and neuroplasticity for infants with or at high risk for cerebral palsy. Developmental Medicine & Child Neurology. 2025 Aug 22
5. Hadders-Algra M. Early diagnosis and early intervention in cerebral palsy. Frontiers in Neurology. 2014 Sep 24;5(185).
6. Spittle AJ, Morgan C. Early Intervention for Children with Cerebral Palsy. Cerebral Palsy. 2025;197–205.
7. te Velde A, Morgan C, Finch-Edmondson M, McNamara L, McNamara M, Paton MCB, et al. Neurodevelopmental Therapy for Cerebral Palsy: A Meta-analysis. Pediatrics. 2022 May 24;149(6).
8. Damiano D, Novak I. Bobath, NeuroDevelopmental Therapy, and clinical science: Rebranding versus rigor. Developmental Medicine & Child Neurology. 2024 Jan 12;66(5):668–8.
9. Damiano DL, Longo E. Early intervention evidence for infants with or at risk for cerebral palsy: an overview of systematic reviews. Developmental Medicine and Child Neurology [Internet]. 2021 Jul 1;63(7):771–84. Available from: https://pubmed.ncbi.nlm.nih.gov/33825199/
10. Morgan C, Novak I, Dale RC, Badawi N. Optimising motor learning in infants at high risk of cerebral palsy: a pilot study. BMC Pediatrics. 2015 Apr 1;15(1).
11. Benfer KA, Whittingham K, Ware RS, Asis Kumar Ghosh, Chowdhury S, Golam Moula, et al. Efficacy of Early Intervention for Infants With Cerebral Palsy in an LMIC: An RCT. Pediatrics. 2024 Mar 22;153(4).
12. Contemporarypediatrics.com. 2024 [cited 2026 May 15]. Available from: https://www.contemporarypediatrics.com/view/effect-of-goals-activity-motor-enrichment-in-infants-with-cerebral-palsy
13. Shierk A, Thias BB, Becker H, Allen B, Chaiprasert B, Lampe KC, et al. Defining Goal-Directed Training for Children with Cerebral Palsy: A Scoping Review and Framework for Implementation. Children. 2025 Aug 8;12(8):1039–9
14. Li Z, So KF, Chen L, Feng S, Hu Y, Cheng L, et al. Clinical guideline for neurorestorative therapy in pediatric cerebral palsy (2024 China version). Journal of Neurorestoratology [Internet]. 2025 Dec;13(6):100253. Available from: https://www.sciencedirect.com/science/article/pii/S2324242625000750?utm_source
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. British Medical Journal [Internet]. 2021;372(71). Available from: https://www.bmj.com/content/372/bmj.n71
16. Tricia Arella Taslim, Dhana Carissa Wangjaya, Deyra Maheswari Wistara, Febry Liana, Fadhlurrahman Ahmad Ariq Bachtiar. Early Goal Directed Training vs Passive Therapy for Functional Independence of Infants with Cerebral Palsy: Systematic Review and Meta-Analysis. PROSPERO 2026 CRD420261394788. Available from https://www.crd.york.ac.uk/PROSPERO/view/CRD420261394788.
17. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: A revised Cochrane risk-of-bias tool for randomized trials. 2019.
18. McGuinness LA, Higgins JPT. robvis: An R package and web app for visualising risk-of-bias assessments. Syst Rev. 2020;9(1):376.
19. McGuinness, LA, Higgins, JPT. Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Res Syn Meth. 2020; 1- 7. https://doi.org/10.1002/jrsm.[1411]
20. Morgan C, Novak I, Dale RC, Guzzetta A, Badawi N. Single blind randomised controlled trial of GAME (Goals - Activity - Motor Enrichment) in infants at high risk of cerebral palsy. Research in Developmental Disabilities. 2016 Aug;55:256–67.
21. Eliasson AC, Nordstrand L, Ek L, Lennartsson F, Sjöstrand L, Tedroff K, et al. The effectiveness of Baby-CIMT in infants younger than 12 months with clinical signs of unilateral cerebral palsy; an explorative study with randomized design. Research in Developmental Disabilities. 2018 Jan;72:191–201.
22. Boyd RN, Greaves S, Ziviani J, Novak I, Badawi N, Pannek K, et al. Randomized Comparison Trial of Rehabilitation Very Early for Infants with Congenital Hemiplegia. The Journal of Pediatrics. 2024 Oct 1;114381–1.
23. Benfer KA, Whittingham K, Ware RS, Asis Kumar Ghosh, Chowdhury S, Golam Moula, et al. Efficacy of Early Intervention for Infants With Cerebral Palsy in an LMIC: An RCT. Pediatrics. 2024 Mar 22;153(4).
24. Armstrong EL, Boyd RN, Horan SA, Kentish MJ, Ware RS, Carty CP. Functional electrical stimulation cycling, goal-directed training, and adapted cycling for children with cerebral palsy: a randomized controlled trial. Developmental Medicine & Child Neurology. 2020 Aug 9;62(12).
25. Choudhary A, Gulati S, Kabra M, Singh UP, Sankhyan N, Pandey RM, et al. Efficacy of modified constraint induced movement therapy in improving upper limb function in children with hemiplegic cerebral palsy: A randomized controlled trial. Brain and Development. 2013 Oct;35(9):870–6.
26. Hadders-Algra M. Early diagnosis and early intervention in cerebral palsy. Frontiers in Neurology. 2014 Sep 24;5(185).
27. Kleim JA, Jones TA. Principles of Experience-Dependent Neural Plasticity: Implications for Rehabilitation After Brain Damage. Journal of Speech, Language, and Hearing Research. 2008;51(1).
28. McIntyre S, Goldsmith S, Webb A, Ehlinger V, Hollung SJ, McConnell K, et al. Global Prevalence of Cerebral Palsy: a Systematic Analysis. Developmental Medicine & Child Neurology [Internet]. 2022 Aug 11;64(12):1494–506. Available from: https://onlinelibrary.wiley.com/doi/10.1111/dmcn.[15346]
29. Morgan C, Badawi N, Boyd RN, Spittle AJ, Dale RC, Kirby A, et al. Harnessing neuroplasticity to improve motor performance in infants with cerebral palsy: a study protocol for the GAME randomised controlled trial. BMJ Open. 2023 Mar;13(3):e070649.
30. Huroy M, Behlim T, Andersen J, Buckley D, Fehlings D, Kirton A, et al. Stability of the Gross Motor Function Classification System over time in children with cerebral palsy. Developmental Medicine & Child Neurology. 2022 Aug 8;64(12):1487–93.

31. Morgan C, Fetters L, Adde L, Badawi N, Bancale A, Boyd RN, et al. Early Intervention for Children Aged 0 to 2 Years With or at High Risk of Cerebral Palsy: International Clinical Practice Guideline Based on Systematic Reviews. JAMA Pediatrics [Internet]. 2021 May 17;175(8). Available from: https://jamanetwork.com/journals/jamapediatrics/article-abstract/2780012

32. Omrani M, Rosaria Rita Chiarelli, Acquaviva M, Bassani C, Gloria Dalla Costa, Montini F, et al. Machine learning-driven diagnosis of multiple sclerosis from whole blood transcriptomics. Brain Behavior and Immunity [Internet]. 2024 Oct 1 [cited 2024 Aug 7];121:269–77. Available from: https://pubmed.ncbi.nlm.nih.gov/39097200/

33. Fabrizia Festante, Antonelli C, Mazzotti S, Guzzetta A. Early Intervention in Cerebral Palsy: From Theory to Current Practice. Springer eBooks. 2023 Jan 1;149–92.

34. Chorna O, Heathcock J, Key A, Noritz G, Carey H, Hamm E, et al. Early childhood constraint therapy for sensory/motor impairment in cerebral palsy: a randomised clinical trial protocol. BMJ Open [Internet]. 2015 Dec;5(12):e010212. Available from: https://bmjopen.bmj.com/content/5/12/e010212

35. Novak I, Morgan C, Adde L, Badawi N, Block S, Boyd RN, et al. Early, Intensive, Active, and Specific: Fractional Dose of Goal-Directed, Activity-Based Early Intervention for Infants at High Risk of Cerebral Palsy—A Systematic Review. J Clin Med. 2023;12(11):3621.

36. Novak I, Morgan C, McNamara L, Te Velde A, Badawi N, Bimanual and Constraint-Induced Movement Therapy Team. Baby-CIMT for infants with asymmetric brain lesions: a randomized controlled trial. Dev Med Child Neurol. 2022;64(9):1112-1121.

DOI: 10.52629/jamsa.v12i1.1105

Supplementary Files

Comparative Efficacy and Safety of Dual Orexin Receptor Antagonists (DORAs) Versus Z-Drugs for Insomnia in Older Adults : A Systematic Review

DOI: 10.52629/jamsa.v12i1.1105

Appendix 1. Search Strategy

Database	Keywords	Records Retrieved
PubMed	(("Cerebral Palsy"[MeSH Terms] OR "Cerebral Palsy" OR "High Risk Infant" OR "High Risk Infants")) AND (("Goal-Directed Training" OR "goal-directed" OR "activity-based" OR "GAME intervention" OR "Small Step" OR "task-oriented")) AND (("PEDI" OR "PEDI-CAT" OR "functional independence" OR "functional outcome"))	19
ScienceDirect	("Cerebral Palsy" OR "High Risk Infant") AND ("goal directed" OR "activity-based" OR "task-oriented" OR "GAME intervention") AND ("PEDI" OR "functional outcome")	299
WILEY	(Abstract:"Cerebral Palsy" OR Abstract:"high risk infant") AND (Abstract:"goal directed" OR Abstract:"activity-based" OR Abstract:"GAME") AND (Abstract:"PEDI" OR Abstract:"functional")	12
ProQuest	noft(("Cerebral Palsy" OR "High Risk Infant") AND ("goal directed" OR "activity-based" OR "task-oriented" OR "GAME intervention") AND ("PEDI" OR "functional outcome"))	232
MedRXiv	"Cerebral Palsy" AND "goal-directed" AND "infant"	10

JAMSA Journal of Asian Medical Students' Association

Efficacy of Complementary Nutritional and Herbal Interventions on Peripheral Inflammatory Biomarkers and Clinical Outcomes in Parkinson's Disease: A Systematic Review

Background: Parkinson's disease is a progressive neurodegenerative illness associated with dopaminergic neurone loss, neuroinflammation, oxidative stress, and disturbance of the gut flora. While findings are still inconsistent, probiotics and synbiotics are examples of non-pharmacological adjuvant therapies that have demonstrated potential benefits through regulation of neurotrophic and inflammatory pathways.

Objective: This review was conducted to evaluate the impact of non-pharmacological adjuvant therapy, especially complementary nutritional interventions and herbal agents, such as probiotics, Omega-3 fatty acids, antioxidants/Vitamin E, dietary supplements, and herbal or traditional medicines, on systemic inflammatory biomarkers, neurotrophic factors, metabolic pathways, and clinical outcomes in patients with PD.

Methods: Based on PRISMA 2020 guidelines, this study was conducted and the protocol was registered in PROSPERO (CRD420261395485). Literature from PubMed, ProQuest, ScienceDirect, Springer Nature, and Cochrane Library was screened according to inclusion and exclusion criteria. A total of four RCT were included in the analysis.

Results: Four RCT on probiotics, synbiotics, Zishen Pingchan Granule, and vitamin D3 supplementation in Parkinson's disease were included. The studies showed improvements in inflammatory and neuroprotective biomarkers such TNF-α, IL-6, CRP, BDNF, and α-synuclein, as well as selected motor and non-motor outcomes. No significant treatment-related adverse effects were regularly reported.

Conclusion: Complementary nutrition and herbal therapy could provide anti-inflammatory and neuroprotective benefits in Parkinson's disease. However, the current information available is limited by small sample numbers and methodological heterogeneity. Confirmation of the clinical effectiveness and long-term therapeutic promise requires more standardised studies.

Keywords: Parkinson's disease, Placebo, Non-Pharmacological interventions, Inflammatory biomarkers, Neuroprotective biomarkers

Nicky Stevani Wijaya[1], Marcella[1], Alicia Priscila Estelle[1], Putu Ayu Anindita Maheswari Kusumaningtyas Nugraha Budha[1], Joyceline Chika Tanely[1]

[1]Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

Category: Review Articles

Date Received: 17 May 2026

Date Accepted: 03 July 2026

Correspondence to:

Nicky Stevani Wijaya

Atma Jaya Catholic University of Indonesia

wijayanicky2@gmail.com

Introduction

As the fastest-growing neurodegenerative condition globally, Parkinson's disease (PD) represents a chronic and advancing neurological impairment. At the microscopic level, its hallmark pathology is defined by the selective degeneration of dopaminergic pathways within the substantia nigra, alongside the abnormal intracellular accumulation of α-synuclein proteins, which aggregate to form Lewy bodies. From an epidemiological perspective, PD demonstrates a point prevalence of approximately 1 to 2 cases per 1,000 individuals across the general population. The incidence exhibits a sharp, age-dependent trajectory; it comprises roughly 1% of cohorts exceeding 60 years of age, and escalates further to encompass 2% to 3% of elderly populations aged 65 and older. While the vast majority of cases occur sporadically, an underlying hereditary genetic susceptibility serves as the primary etiologic driver in 5% to 10% of the affected patient population.[1]

Crucial motor manifestations of PD, encompassing tremors, rigidity, akinesia, hypokinesia, and bradykinesia, stem directly from a profound dopaminergic deficit within the posterior putamen, leading to subsequent disruption of the central motor circuitry.[2] Beyond its characteristic motor impairments, PD widely compromises non-motor neurological pathways. For instance, dopaminergic depletion within the caudate nucleus, coupled with architectural disruptions in associative circuits, frequently triggers early-onset executive dysfunction, while degeneration of central noradrenergic and serotonergic networks serves as a primary driver for neuropsychiatric manifestations, including severe apathy, generalized anxiety, and depressive mood shifts.[2]

Significant pathological involvement, such as degeneration in the locus coeruleus, raphe nuclei, hypothalamus, and autonomic nervous system structures, may explain the emergence of non-motor symptoms that are often unresponsive to dopamine replacement therapy. Some interconnected mechanisms, such as α-synuclein aggregation, mitochondrial dysfunction, impaired ubiquitin-proteasome and autophagy-lysosome systems, and chronic neuroinflammation, lead to progressive neuronal degeneration in PD.[3]

Given the important role of these mechanisms, particularly neuroinflammation, in disease progression, several non-pharmacological adjuvant therapies have shown potential therapeutic effects in PD through anti-inflammatory, antioxidant, and neuroprotective pathways, including probiotics, synbiotics, Chinese herbal medicine, and vitamin D3, which have been developed. Probiotic therapy has therapeutic and preventive effects via a variety of pathways, including competition for nutrients and binding to the intestinal epithelium, antagonism against pathogenic microbes, and metabolic interactions within the gut microbiota.[4] Besides probiotics, synbiotic therapy could also improve clinical results in PD by modulating the inflammation, oxidative stress, and neurotrophic pathways.[5]

Furthermore, Chinese traditional medicine has shown potential therapeutic effects in PD patients with depression by modulating neurotrophic and inflammatory pathways.[6] Besides Chinese traditional medicine, vitamin D3 supplementation has shown potential therapeutic effects in PD by reducing inflammation and improving functional performance. Patients with PD often have vitamin D insufficiency, which may correlate with increased inflammation and a higher risk of falling.[7]

PD has been treated using some types of non-pharmacological adjuvant therapy that target inflammatory pathways. Nevertheless, choosing the most effective supplementary intervention remains difficult due to variations in mechanisms of action, clinical efficacy, biomarker modification, intervention length, and patient responses among regimens. Some non-pharmacological adjuvant therapies, such as probiotics and synbiotics, have demonstrated potential therapeutic effects by modulating systemic inflammatory markers and enhancing motor and non-motor clinical features of PD. Nonetheless, there are variations across studies regarding the magnitude of the amplitude of biomarker modulation and the depth of clinical improvement as measured by Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) scores, depression measures, and quality-of-life assessments. In addition, most previous studies focused on evaluating a single intervention or comparing it to a placebo, with only a few studies comprehensively evaluating the relationship between inflammatory biomarkers, neurotrophic factors, metabolic signaling pathways, and clinical outcomes in PD patients. Therefore, this systematic review was conducted to evaluate the impact of non-pharmacological adjuvant therapy on systemic inflammatory biomarkers, neurotrophic factors, metabolic pathways, and clinical manifestations in patients with PD.

Method

Protocol and Registration

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[8] Prior to conducting the study, the research protocol was prospectively registered and archived in the International Prospective Register of Systematic Review (PROSPERO) under the registration identifier CRD420261395485.

Eligibility Criteria

Study inclusion was strictly governed by a structured PICO framework designed to isolate relevant clinical data. The target population was restricted to patients with a confirmed diagnosis of Parkinson's disease. The interventions included non-pharmacological adjuvant therapies, especially complementary nutritional interventions and herbal agents, such as probiotics, Omega-3 fatty acids, antioxidants/Vitamin E, dietary supplements, and herbal or traditional medicines. Comparators included placebo, standard Parkinson's care (Standard of Care/SoC) without adjuvant therapy, or patients' baseline measurements, depending on the study design.

The investigative endpoints were divided into primary and secondary categories. The primary focus centered on peripheral biomarker alterations, which included down-regulated systemic inflammatory markers (namely TNF-α, IL-6, and IL-1), metabolic signaling shifts involving low-density lipoprotein receptor (LDLR) and peroxisome proliferator-activated receptor gamma (PPAR-γ), and enhanced expression of neurotrophic factors such as brain-derived neurotrophic factor (BDNF). Conversely, secondary parameters evaluated objective clinical changes in motor and non-motor symptom presentation, specifically tracking fluctuations in MDS-UPDRS metrics, psychometric depression scales, and standardized quality-of-life evaluations. To capture a comprehensive body of evidence, the protocol accepted randomized controlled trials (RCTs) because RCTs are considered to provide stronger evidence.

Sources and Search Strategy

A comprehensive literature search was performed across five databases: PubMed, ProQuest, ScienceDirect, Springer Nature, and Cochrane Library, covering all records from database inception to March 2026. The search strategy (Appendix 1) was developed using eight core keywords: Parkinson disease, dietary supplement, probiotics, complementary therapies, adjuvant therapy, inflammation, cytokines, biomarkers. Both Medical Subject Headings (MeSH) and free-text terms were employed and combined using Boolean operators to maximise retrieval sensitivity.

In addition to the database search, a snowballing technique was employed to identify further relevant studies. This involved screening the reference lists of all included articles (backward snowballing) as well as identifying studies that cited the included articles (forward snowballing). This approach was undertaken to ensure that no potentially eligible studies were overlooked. Reference management and duplicate removal were performed systematically before screening.

Selection Process

Study selection was conducted using a two-stage screening process comprising an initial screening of titles and abstracts and a full-text review to determine final eligibility. This approach was undertaken to ensure objectivity and consistency throughout the selection process. Any disagreements were resolved through discussion and consensus. The entire selection procedure was documented in accordance with the PRISMA 2020 guidelines, detailing the number of records identified, screened, excluded, and included in the final analysis.

Data Collection Process

Data extraction included study characteristics (author, year, country, and study design), sample characteristics (sample size), eligibility criteria for PD, intervention and comparator regimens, and outcomes of interest, including changes in inflammatory cytokines (TNF-α, IL-1, IL-6), neurotrophic factors (BeDNF), metabolic pathway markers (PPAR-γ and LDLR), motor symptom severity, depression scores, and quality-of-life measures in patients with PD.

Risk of Bias Assessment

The risk of bias in the included studies was assessed using the Cochrane Risk of Bias 2 (RoB 2) tool.[9] This tool evaluates five domains of bias, including bias arising from the randomization process, bias due to deviations from intended intervention, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. The assessment was conducted independently by two

reviewers to ensure objectivity and consistency. Each domain was judged as 'low risk', 'some concerns', or 'high risk' of bias, in accordance with the RoB 2 guidance. Any disagreements between the reviewers were resolved through discussion and consensus, with consultation from a third reviewer where necessary.

As shown in Figure 2, no study was judged to have a high risk of bias. Most studies showed a low risk of bias in the domains related to the randomization process (D1), deviations from intended interventions (D2), and outcome measurement (D4). However, all included studies were rated as having some concerns in the domains of missing outcome data (D3) and selection of the reported result (D5). These judgements primarily reflected insufficient reporting regarding the handling of missing data and the lack of clear evidence that the reported outcomes were prespecified in a published protocol or statistical analysis plan. Consequently, the overall risk of bias for all included studies was judged as some concerns, in accordance with the RoB 2 guidance.

Result

Study Selection

A total of 1,373 records were identified through database searching. After removing duplicates and records marked as ineligible by automation tools, 183 studies remained for title and abstract screening. A total of 179 records were excluded due to the wrong population, intervention, comparison, and outcomes. Ultimately, 4 studies were included in this systematic review

Study Characteristics

The included studies consisted of four randomized controlled trials (RCTs) conducted between 2023 and 2025 across several countries, such as the United Kingdom, Sweden, China, and Poland. All studies involved patients diagnosed with PD, with varying participant characteristics based on disease stage, age range, and specific inclusion criteria. Most studies involved adults with mild to moderate PD, while one

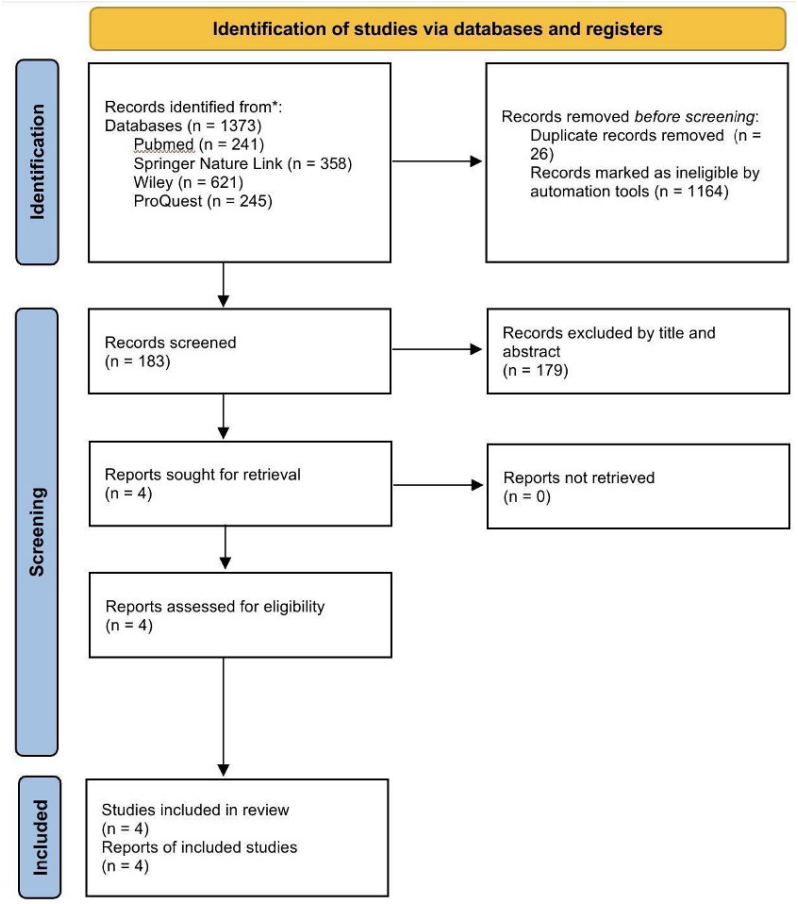

Figure 1: Diagram Flow of Literature Search Strategy

study specifically included patients with PD and comorbid depression. Participant sample sizes ranged from 40 to 74 participants.

A variety of non-pharmacological adjuvant interventions were assessed, including probiotics, synbiotics, traditional Chinese herbal medicine, Zishen Pingchan Granule (ZPG), and Vitamin D3 supplementation. Comparator groups generally received placebo treatment alongside standard Parkinson's care. The assessed outcomes primarily focused on inflammatory and neurotrophic biomarkers, including TNF-α, IL-1β, IL-6, CRP, BDNF, α-synuclein, and oxidative stress markers, as well as clinical outcomes, including changes in MDS-UPDRS, Hamilton Depression Scale (HAMD), and functional status scores. Several studies also explored alterations in gut microbiota composition and systemic inflammatory regulation following intervention.

Overall, the interventions showed typically positive safety profiles, with most studies reporting no serious adverse reactions. Some trials noted that the frequency of adverse events was comparable between intervention and placebo groups, while others reported no significant treatment-related complications. Although the included studies consistently suggested potential benefits of complementary nutritional and herbal therapies in improving inflammatory profiles and clinical symptoms in PD, direct comparisons across studies remain limited because of differences in intervention types, biomarker assessments, follow-up duration, and outcome reporting methods.

The methodological quality and overall risk of bias varied across the selected literature. Methodological evaluations indicated that a majority of the included trials exhibited a robust design with a low risk of bias within three primary evaluation areas: the initial randomization process (D1), adherence to intended therapeutic interventions (D2), and the objective measurement of clinical outcomes (D4). Specifically, Leta et al. (2025)[10] and Bytowska et al. (2023)[7] were generally assessed as having low risk in D1, D2, and D4, although some concerns remained regarding missing outcome data (D3) and selection of the reported result (D5). Similarly, Ning et al. (2023)[6] showed low risk in deviations from intended interventions (D2) and outcome measurement (D4), but some concerns were identified in the randomization process (D1), missing outcome data (D3), and selective reporting (D5).

Ramadan et al. (2025)[5] demonstrated low risk in the

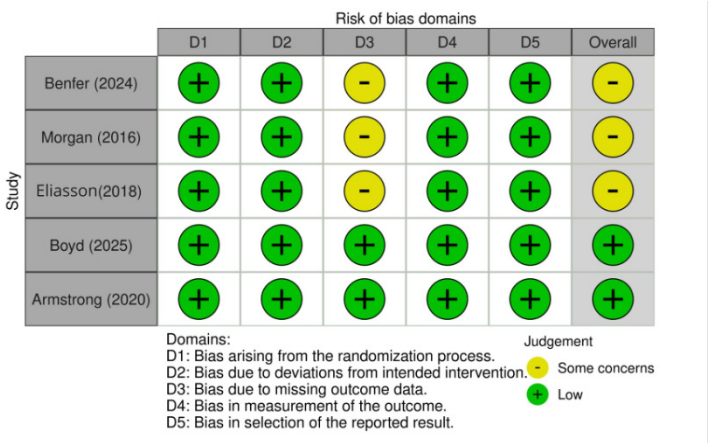

Figure 2: Risk of Bias domains of RCT studies

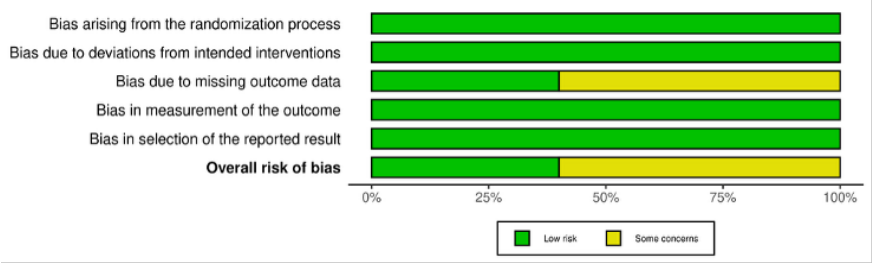

Figure 3: Overall risk of bias of RCT studies

Table 1. Study Characteristics of Included Studies

Authors (Year)	Country	Study Design	N (groups)	Population	Intervention vs Control	Key Outcomes	Adverse Events
Leta et al. (2025)[10]	UK and Sweden	RCT	74 (35 vs 39)	Adults ≥18 with MDS-diagnosed PD	Probiotic vs Placebo	Gut microbiota change at 12 wks (primary); inflammatory markers (TNF-α, IFN-γ, IL-6, IL-8, IL-10)	11 events in each arm; 3 vs 2 dropouts due to AEs
Ramadan et al. (2025)[5]	UK	RCT	66 (33 vs 33)	Adults 45–65, de novo PD, Hoehn-Yahr stage 1–4	Synbiotic vs Placebo	MDS-UPDRS motor/non-motor scores (primary); TNF-α, MDA, BDNF, α-synuclein	NR
Ning et al. (2023)[6]	China	RCT	80 (40 vs 40)	Adults 40–80, PD (Hoehn-Yahr 1–4) with mild-moderate depression (HAMD 8–24).	ZPG vs Placebo	HAMD and UPDRS-III scores; BDNF, IL-1β, IL-6, CRP, TNF-α	No serious adverse reactions
Bytowska et al. (2023)[7]	Poland	RCT	42 (21 vs 21)	PD patients on STN-DBS, no prior vitamin D supplementation, no major comorbidities.	Vitamin D3 vs Placebo	Serum vitamin D levels; motor/mobility function; systemic inflammation	NR

RCT, randomized controlled trial; PD, Parkinson's disease; MDS, Movement Disorder Society; MDS-UPDRS, Movement Disorder Society–Unified Parkinson's Disease Rating Scale; HAMD, Hamilton Depression Rating Scale; BDNF, brain-derived neurotrophic factor; TNF-α, tumor necrosis factor-alpha; IFN-, interferon-γ gamma; IL-1β/6/8/10, interleukin-1beta/6/8/10; CRP, C-reactive protein; MDA, malondialdehyde; α-Syn, alpha-synuclein; ZPG, Zishen Pingchan Granule; STN-DBS, subthalamic nucleus deep brain stimulation; N, total sample size; AE, adverse event.

randomization process (D1), while several concerns remained in domains related to deviations from intended interventions (D2), missing outcome data (D3), outcome measurement (D4), and selection of the reported result (D5). Across all included studies, the most common concerns were associated with missing outcome data (D3) and selective reporting (D5), as all studies showed some concerns in these domains.

Overall, the included studies were predominantly judged as having "some concerns" rather than a high risk of bias. The summary risk-of-bias assessment demonstrated that most studies had low risk in randomization, intervention deviations, and outcome measurement domains, whereas concerns related to incomplete outcome data and reporting bias remained relatively common across the included trials.

Discussion

Principal Findings and Clinical Signal

The evidence from four randomised controlled trials (RCTs) assessing complementary nutritional and herbal interventions–such as probiotic supplementation, synbiotic therapy, traditional Chinese herbal medicine (Zishen Pingchan Granule), and vitamin D3 supplementation–in patients with PD was compiled in this systematic review. Together, these studies evaluated biological and clinical results, focusing on neurotrophic markers, oxidative stress markers, peripheral inflammatory biomarkers, and validated clinical scales measuring the burden of motor and non-motor symptoms.[5,6,7,10]

Complementary nutritional and herbal therapies may have potentially helpful anti-inflammatory and neuroprotective benefits in PD, according to the collected research. Several interventions were linked to favourable modulation of systemic inflammatory markers across the reviewed studies. These included improvements in oxidative stress parameters and neurotrophic markers like brain-derived neurotrophic factor (BDNF), as well as decreases in pro-inflammatory cytokines like TNF-α, interleukin-1 beta (IL-1ß), interleukin-6 (IL-6), and C-reactive protein (CRP). Modulation of disease-relevant biomarkers, such as alpha-synuclein, has also been identified in several studies, indicating potential mechanistic significance beyond general systemic inflammation. In some trials, these biomarker changes were accompanied by improvements in selected clinical outcomes, including MDS-UPDRS scores and depressive symptom measures. However, these findings were not consistent across all included studies. [5,6,7,10]

Despite these positive results, the research as a whole is still preliminary and should be evaluated carefully. Significant methodological heterogeneity, such as variations in intervention type, participant characteristics, biomarker selection, outcome measuring techniques, and follow-up duration, limits direct comparison between trials. Additionally, the very small sample sizes in each of the included trials limit the generalizability of results and lower statistical power. Therefore, there is currently insufficient evidence to conclude that complementary nutritional and herbal interventions provide effective supportive treatment for PD. While some studies suggest possible biological activity, these findings require confirmation in larger, well-designed clinical trials. [5,6,7,10]

Peripheral Biomarkers and Parkinson's Pathophysiology

PD is becoming more well acknowledged as a condition involving systemic immune dysregulation and persistent neuroinflammation in addition to dopaminergic neuronal loss. Peripheral inflammatory indicators, which indicate interactions between systemic inflammation, blood-brain barrier failure, and neurodegenerative progression, may offer clinically relevant insight into underlying disease processes, even though PD pathology is centered in the central nervous system.[5,7]

TNF-α is a pro-inflammatory cytokine that has been closely linked to neuroinflammation associated with PD. Through oxidative stress, mitochondrial malfunction, and apoptotic signaling, activated microglia release TNF-α, which contributes to a persistent inflammatory milieu that causes dopaminergic neuronal damage. Therefore, persistent elevation of TNF-α may be more indicative of persistent neurotoxic inflammatory activity than temporary systemic immune activation.[5]

IL-6 and IL-1ß are important mediators in inflammatory amplification pathways. While IL-6 is linked to chronic inflammatory signalling and may help breach the blood-brain barrier, giving peripheral immune mediators more access to the central nervous system, IL-1ß contributes to microglial activation and neuronal injury. Therefore, elevated levels of these cytokines in the blood may be biologically plausible markers of the inflammatory burden associated with a disease.[6]

On the other hand, rather than being a cytokine with a

specific mechanism, C-reactive protein (CRP) is a more general indicator of systemic inflammation. Despite being nonspecific, CRP has significant translational benefits because of its known correlations with PD risk, disease severity, and prognosis, as well as its standardized testing techniques and reliability. According to a recent viewpoint, CRP may be especially helpful in PD biomarker research because it measures chronic inflammatory burden independent of the specific inflammatory pathway involved, which makes it helpful in a variety of neurodegenerative conditions where the exact mechanisms are still unclear.[11]

Neuroprotection-related markers need to be taken into account as well. Brain-derived neurotrophic factor (BDNF) is essential for maintaining the dopaminergic system, synaptic plasticity, and neuronal survival. While treatments that raise BDNF may represent the restoration of neuroprotective capacity rather than just the suppression of inflammation, decreased BDNF levels have been connected to poor neuroplasticity and the advancement of disease.[5,6]

Given their crucial involvement in PD pathogenesis through protein misfolding and aggregation, more disease-specific biomarkers like α-synuclein offer greater pathological significance. However, the lack of consistency between research, biological compartment variations, and testing methodology heterogeneity continue to make it methodologically difficult to interpret peripheral α-synuclein findings.[5,6]

When considered collectively, peripheral biomarker modulation should be taken as potentially significant evidence of changed neuroimmune and neurodegenerative processes rather than as a single biochemical fluctuation. Therapeutic approaches that target inflammation and neuroprotection in PD have biologically reasonable support, although various biomarkers vary in their mechanistic specificity and clinical relevance. [5,6]

Methodological Heterogeneity and Interpretation Challenges

One of the major challenges in interpreting the current evidence is not necessarily therapeutic failure, but significant methodological variability among studies. The included trials encompassed a diverse array of interventions, such as probiotics, synbiotics, traditional Chinese herbal medicines, and vitamin D3 supplementation. These interventions are biologically different and operate via various mechanisms. Probiotics and synbiotics mainly influence gut microbiota composition and immune signaling along the gut-brain axis, while herbal formulations primarily demonstrate anti-inflammatory and antioxidant effects via phytochemical pathways.[10] In comparison, vitamin D3 acts as an endocrine immunomodulator that plays a role in neuroimmune regulation and the survival of neurons. Due to the mechanistic differences among these therapies, making direct comparisons between studies is challenging.[7]

Significant diversity was also noted in the characteristics of participants. The studies varied in terms of disease stage, age range, duration of disease, and the existence of comorbid conditions like depression. These variations are clinically significant because PD demonstrates considerable biological diversity, encompassing differences in inflammatory activity, levels of oxidative stress, and the course of neurodegeneration. As a result, therapeutic reactions can vary greatly among different patient subgroups, restricting the general applicability and comparability of results across various studies.[5,6,7,10]

An additional significant constraint was the variety of outcomes evaluated. Research assessed multiple biomarkers and clinical outcomes, such as TNF-α, IL-1ß, IL-6, markers of oxidative stress, α-synuclein, BDNF, gut microbiota diversity, MDS-UPDRS scores, and scales for depression.[10] The lack of standardized outcome measures hindered significant apple-to-apple comparisons among trials. Moreover, various studies had comparatively brief follow-up periods, which might have been inadequate to identify lasting biomarker changes or extended clinical outcomes in a progressive neurodegenerative condition like PD.[5,6,7,10]

Collectively, the observed inconsistency among studies might indicate methodological fragmentation instead of genuine therapeutic ineffectiveness. The differences in intervention methods, participant traits, outcome metrics, and follow-up lengths make it difficult to interpret the overall evidence. Future studies would gain from enhanced methodological standardization, involving more uniform patient stratification, aligned biomarker evaluations, and extended follow-up durations to boost comparability and reinforce conclusions about complementary nutritional and herbal treatments in PD.[5,6,7,10]

Microbiota-Targeted Interventions and the Gut-Brain Axis

The positive outcomes observed with microbiota-targeted treatments in this analysis may be explained biologically by the gut-brain axis, which has emerged as a compelling framework for understanding the pathophysiology of PD. Magistrelli's hypothesis is a well-known idea that suggests PD pathology may start outside the central nervous system, perhaps in the gastrointestinal tract or olfactory system, before gradually moving to the brain. This theory is based on the finding that aberrant α-synuclein aggregation, a clinical characteristic of PD, has been found in enteric nervous tissue and may spread to the substantia nigra and other parts of the brain via neural routes like the vagus nerve. This idea is in line with the clinical finding that gastrointestinal symptoms, especially constipation, frequently appear years before motor symptoms do.[12]

According to this theory, gut microbial dysbiosis may accelerate PD through inflammatory and immunological pathways. Changes in the composition of the gut microbiota may compromise the integrity of the intestinal barrier, enabling the translocation of bacterial endotoxins like lipopolysaccharide into the systemic circulation. This can worsen neuroinflammatory processes through immune signalling and disruption of the blood-brain barrier, as well as peripheral inflammation. The positive outcomes seen in the probiotic and synbiotic therapies covered in this review may be explained by this. The therapeutic potential of microbiota-targeted interventions as supplemental strategies in PD management is reinforced by recent clinical evidence showing that probiotic supplementation in PD patients significantly reduced IL-6 levels while improving motor and non-motor symptoms, including gastrointestinal symptom burden.[12]

Despite high biological plausibility, there is currently inadequate data to show a direct causal association between microbiome modulation and disease modification in PD. Comparability between research is still hampered by differences in probiotic strains, intervention duration, participant characteristics, and outcome-measuring techniques. However, more research into microbiota-directed treatments within deeper neuroprotective approaches is encouraged by the developing molecular connection between gut dysbiosis, chronic inflammation, and neurodegeneration.[5,6,7,10,12]

Clinical Implications of Complementary Interventions

The results of this review indicate that adjuvant nutritional and herbal therapies could provide beneficial clinical support for individuals with PD, especially by influencing inflammation, oxidative stress, and gut-brain axis mechanisms. Multiple studies showcased enhancements in peripheral inflammatory markers, neurotrophic factors, depressive symptoms, and specific motor or non-motor clinical results. Their results suggest that such strategies may aid in improving inflammatory balance, assist in symptom management, boost mood and psychological well-being, and possibly lead to an enhanced quality of life for certain patients with PD.[5]

Nevertheless, existing evidence is still inadequate to endorse these therapies as substitutes for recognized PD treatments. Conventional pharmacological treatments, such as levodopa and dopamine agonists, continue to be the foundation of PD management due to their proven effectiveness in managing motor symptoms and preserving functional ability. Conversely, the complementary interventions assessed in this review were typically examined in limited groups, over brief follow-up durations, and utilizing varied methodologies, restricting the robustness and applicability of the findings.[5,6,7,10]

Thus, these nutritional and herbal methods should presently be regarded as complementary supportive strategies instead of disease-modifying or independent treatments. Their possible worth may be in enhancing traditional treatment by addressing systemic inflammation, oxidative stress, gastrointestinal issues, and the burden of non-motor symptoms, aspects that are frequently insufficiently managed by dopaminergic therapy alone. Larger and more standardized clinical trials are still necessary before these interventions can be included in standard evidence-based PD treatment guidelines.[5,6,7,10]

Safety and Tolerability

The overall positive safety profile of the included alternative nutritional and herbal therapies is a significant finding of this review. No significant treatment-related side effects were consistently observed across the included randomized controlled trials, indicating that these interventions may be fairly well tolerated as supplemental therapies in PD patients. While no serious safety issues were seen with

herbal or vitamin D supplementation, probiotic and synbiotic therapies showed adequate tolerability, with adverse event frequencies equivalent to control groups in several studies.[5,6,7,10]

Tolerability is an important factor to take into account when assessing supplementary therapy methods because patients with PD tend to be older individuals with various comorbidities, polypharmacy exposure, and greater vulnerability to medication-related adverse effects. Therefore, complementary therapies that show promise for neuroprotective or anti-inflammatory effects without significantly adding to the burden of treatment may be appealing supportive choices. Specifically, compared to more pharmacologically intensive treatments, microbiota-targeted medicines like probiotics and synbiotics may offer a relatively low-risk intervention strategy.[5,6,7,10]

These results should be interpreted with caution, though, since the information that is now available remains limited by short follow-up periods, small sample numbers, and inadequate long-term safety monitoring. Concerns about formulation standardization, pharmacokinetic variability, and possible herb-drug interactions that might not be completely recorded in short-term clinical studies may also arise with some therapies, especially herbal formulations. Therefore, larger and longer-duration trials are required to better show long-term safety and real-world clinical applicability, even though the current evidence suggests adequate short-term tolerability.[5,6,7,10]

Comparison with Existing Literature, Strengths of This Review, and Limitations

The findings of this review are generally consistent with previous studies investigating probiotics, neuroinflammation, and additional anti-inflammatory treatments in PD. Earlier reviews indicated that interventions aimed at microbiota may enhance inflammatory regulation, gastrointestinal issues, and certain motor or non-motor symptoms by influencing the gut-brain axis and overall immune responses.[5,6,7,10] Research conducted by Kesika et al. (2022) emphasized the increasing significance of neuroinflammation and peripheral inflammatory biomarkers in the pathophysiology of PD, reinforcing the possible importance of immunomodulatory approaches in disease management. This review broadens the existing evidence by combining various types of complementary interventions, such as probiotics, synbiotics, herbal formulations, and vitamin D supplementation, while also assessing both peripheral inflammatory biomarkers and clinical outcomes from recent human interventional studies.

This analysis possesses multiple strengths, such as a targeted clinical question, the inclusion of recent randomized controlled trials, and the combination of biomarker results with clinically significant outcomes like motor symptoms, depressive symptoms, and non-motor effects. Nonetheless, various constraints must also be recognized. The evidence still remains sparse, comprising just four randomized controlled trials and relatively small sample sizes of about 40-74 participants, which diminishes statistical power and generalizability. Considerable methodological diversity among interventions, populations, biomarkers, and outcome metrics also restricted direct comparisons between studies and hindered quantitative meta-analysis. Moreover, the follow-up periods were comparatively brief, potentially rendering them inadequate for evaluating lasting therapeutic or disease-modifying outcomes in a chronic progressive condition like PD. A further significant limitation is that peripheral inflammatory markers, such as TNF-α, IL-6, CRP, and BDNF, might not accurately represent neuroinflammation in the central nervous system and should thus be interpreted carefully when deducing underlying neurodegenerative processes.[5,6,7,10]

Conclusion

This systematic review concludes that current evidence is insufficient to establish the effectiveness of complementary nutritional and herbal interventions, such as probiotics, synbiotics, traditional Chinese herbal medicine, and vitamin D3 supplementation, as supportive treatments for Parkinson's disease. While several studies reported potential mechanisms related to the gut-brain axis and anti-inflammatory, antioxidant, and neuroprotective effects, these findings remain inconclusive due to the limitations and heterogeneity of the included studies. Further high-quality randomized controlled trials are required before definitive conclusions can be drawn. Numerous studies showed improvements in some clinical outcomes, such as motor symptoms, depressive symptoms, and non-motor manifestations, as well as peripheral inflammatory biomarkers, oxidative stress markers, and neurotrophic factors like BDNF. These findings support the increasing understanding that systemic

immune dysregulation and neuroinflammation play significant roles in the etiology of PD.[5,6,7,10]

However, the current data is still limited by small sample sizes, short follow-up periods, diverse interventions, and variations in outcome assessments and participant characteristics. As a result, it is no longer able to draw solid conclusions on clinical efficacy and disease-modifying potential. Currently, these treatments should not be used in place of conventional dopaminergic therapy, but rather as supplemental supporting strategies.[5,6,7,10]

To evaluate the efficacy and long-term safety of supplementary nutritional and herbal therapy in PD patients, future research should focus on more comprehensive, methodologically standardized randomized controlled trials. More consistency in intervention methods, biomarker selection, patient classification, and clinical outcome assessments will increase the overall body of data and improve research comparability. To enhance clinical outcomes and offer substantial neuroprotective or disease-modifying benefits in PD, more study is required to understand whether peripheral inflammation and gut-brain axis functions may be controlled.[5,6,7,10]

Acknowledgement

The authors extend their sincere gratitude to the investigators of the primary literature included in this systematic review for their valuable scientific contributions.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Source of Funding

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Availability of Data and Material

The datasets analyzed during the current systematic review were compiled entirely from previously peer-reviewed publications and open-access repositories. No primary or novel datasets were generated for this study. The extracted clinical data and analytical materials supporting the conclusions of this manuscript remain available from the corresponding author upon reasonable academic request.

Author's Contributions

All authors participated equally in the intellectual design, comprehensive literature search, data extraction, statistical analysis, interpretation of clinical findings, and draft preparation of this manuscript. Furthermore, every individual author carefully reviewed, revised, and granted final approval for the submitted version of the text.

References

1. Zafar S, Yaddanapudi SS. Parkinson Disease [Internet]. Nih.gov. StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470193/
2. Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, et al. Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. The Lancet Neurology [Internet]. 2009 Dec;8(12):1128–39. Available from: https://www.sciencedirect.com/science/article/pii/S1474442209702935
3. Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. Parkinson's Disease: Pathogenesis and Clinical Aspects [Internet]. 2018;1(1):3–26. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536722/
4. Tan AH, Hor JW, Chong CW, Lim S. Probiotics for Parkinson's disease: Current evidence and future directions. JGH Open. 2020 Nov 20;5(4):414–9.
5. Ramadan ME, Mostafa TM, Ghali AA, El-Afify DR. Randomized controlled trial evaluating synbiotic supplementation as an adjuvant therapy in the treatment of Parkinson's disease. Inflammopharmacology. 2025 May 28;33(7):3897–908.
6. Ning H, Zhou H, Yang N, Ren J, Wang H, Liu W, et al. Effect of Zishen pingchan granules combined with pramipexole on serum BDNF, IL-1β, IL-6, CRP, TNF-α levels in depressed patients with Parkinson's disease: Results of a randomized, double-blind, controlled study. Experimental Gerontology. 2023 Oct;182:112295.
7. Bytowska ZK, Korewo-Labelle D, Berezka P, Kowalski K, Przewłócka K, Libionka W, et al. Effect of 12-Week BMI-Based Vitamin D3 Supplementation in Parkinson's Disease with Deep Brain Stimulation on Physical Performance, Inflammation, and Vitamin D Metabolites. International Journal of Molecular Sciences [Internet]. 2023 Jun 15 [cited 2026 May 2];24(12):10200. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10299437/
8. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews BMJ 2021; 372 :n71 doi:10.1136/bmj.n71
9. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898. Published 2019 Aug 28. doi:10.1136/bmj.l4898
10. Leta V, Pavlos Zinzalias, Batzu L, Mandal G, Staunton J, Jernstedt F, et al. Effects of a Four-Strain Probiotic on Gut Microbiota, Inflammation, and Symptoms in Parkinson's Disease: A

Randomized Clinical Trial. Movement Disorders. 2025 Oct 23;

11. Mehta N, Luthra NS, Corcos DM, Fantuzzi G. C-reactive protein as the biomarker of choice to monitor the effects of exercise on inflammation in Parkinson's disease. Front Immunol [Internet]. 2023;14:1178448. Available from: http://dx.doi.org/10.3389/fimmu.2023.1178448

12. Magistrelli L, Contaldi E, Visciglia A, Deusebio G, Pane M, Amoruso A. The impact of probiotics on clinical symptoms and peripheral cytokines levels in Parkinson's disease: Preliminary in vivo data. Brain Sci [Internet]. 2024;14(11):1147. Available from: http://dx.doi.org/10.3390/brainsci14111147

JAMSA Journal of Asian Medical Students' Association

Supplementary Files

Comparative Efficacy and Safety of Dual Orexin Receptor Antagonists (DORAs) Versus Z-Drugs for Insomnia in Older Adults : A Systematic Review

Appendix 1. Search Strategy

PubMed

#1	"Parkinson Disease"[Mesh] OR "Parkinsonian Disorders"[Mesh] OR "Parkinson*"
#2	"Dietary Supplements"[Mesh] OR "Probiotics"[Mesh] OR "Fatty Acids, Omega-3"[Mesh] OR "Vitamin E" OR "Drugs, Chinese Herbal"[Mesh] OR "Complementary Therapies"[Mesh] OR "Adjuvant Therapy"
#3	"Inflammation"[Mesh] OR "Cytokines"[Mesh] OR "Biomarkers"[Mesh] OR "TNF-alpha" OR "Interleukins" OR "PPAR gamma" OR "BDNF"
#4	#1 AND #2 AND #3

Springer Nature Link

#1	"Parkinson Disease"[Mesh] OR "Parkinsonian Disorders"[Mesh] OR "Parkinson*"
#2	"Dietary Supplements"[Mesh] OR "Probiotics"[Mesh] OR "Fatty Acids, Omega-3"[Mesh] OR "Vitamin E" OR "Drugs, Chinese Herbal"[Mesh] OR "Complementary Therapies"[Mesh] OR "Adjuvant Therapy"
#3	#1 AND #2
#4	"Inflammation"[Mesh] OR "Cytokines"[Mesh] OR "Biomarkers"[Mesh] OR "TNF-alpha" OR "Interleukins" OR "PPAR gamma" OR "BDNF"
#4	#3 AND #4

Wiley

#1	(TI,AB,KW:("Parkinson disease" OR "Parkinsonian disorder*" OR Parkinson*))
#2	(TI,AB,KW:("dietary supplement*" OR probiotic* OR "omega-3" OR "omega 3" OR "fatty acid*" OR "vitamin E" OR nutraceutical* OR "Chinese herbal*" OR phytotherap* OR "complementary therap*" OR "adjuvant therap*"))
#3	#1 AND #2
#4	(TI,AB,KW:(inflammat* OR neuroinflammat* OR cytokine* OR biomarker* OR "TNF-alpha" OR TNF OR interleukin* OR "PPAR gamma" OR PPAR ɣ OR BDNF))
#5	#3 AND #4

ProQuest

#1	"Parkinson disease" OR "Parkinsonian disorder*" OR Parkinson*
#2	"dietary supplement*" OR probiotic* OR "omega-3" OR "omega 3" OR "fatty acid*" OR "vitamin E" OR nutraceutical* OR "Chinese herbal*" OR phytotherap* OR "complementary therap*" OR "adjuvant therap*"
#3	#1 AND #2
#4	inflammat* OR cytokine* OR biomarker* OR "TNF-alpha" OR TNF OR interleukin* OR "PPAR gamma" OR PPAR ɣ OR BDNF) NOT ("systematic review" OR "Meta-analysis"
#5	#3 AND #4

DOI: 10.52629/jamsa.v12i1.1106

Molecular Pathogenesis of Valproate-Induced Neural Tube Defects: A Systematic Review of Apoptosis, Folate Pathways, and Gene Expression Dysregulation

Background: Significant morbidity and mortality are related to neural tube defects (NTDs), which result from failure of neural tube closure during early development. Through several teratogenic processes, valproic acid (VPA), a commonly used antiepileptic treatment, is strongly correlated with a higher incidence of NTDs.

Objective: This review attempts to synthesize current research and integrate molecular mechanisms associated with VPA-induced NTDs, which are often studied separately.

Methods: Based on PRISMA 2020 guidelines, this study was conducted, and the protocol was registered in PROSPERO (CRD420261384605). Literature from PubMed, ProQuest, ScienceDirect, and Wiley were screened independently by all authors according to inclusion and exclusion criteria, yielding seven studies for this systematic review. Risk of Bias assessment was conducted using the SYRCLE and Modified SYRCLE's RoB.

Results: The results showed that VPA causes NTDs via a variety of pathways, including oxidative stress, apoptosis, folate-pathway dysregulation, epigenetic modification, and retinoic acid signaling disruption. Genetic susceptibility influenced teratogenic outcomes through differential regulation of folate-related and anti-apoptotic genes, whereas VPA exposure changed the expression of genes involved in neurulation and embryonic development. Additionally, recent studies indicate that by inhibiting apoptotic pathways and reducing the prevalence of NTDs, maternal immune activation can reduce the teratogenic effects of VPA.

Conclusion: Developmental signaling dysregulation and host susceptibility factors interact in a complex manner in VPA-associated teratogenicity. To support better preventive and therapeutic strategies for pregnant individuals in need of VPA treatment, further mechanistic research is required.

Keywords: Valproic Acid, Neural Tube Defects, Folate metabolism, Apoptosis, Epigenetic regulation

Aditya Duta Vanda Wirakusuma[1], Tson Rirutolmo Chen[1], Marcella[1], Katrina Jessica Wijaya[1], Tricia Arella Taslim[1]

[1]Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

Category: Review Articles

Date Received: 16 May 2026

Date Accepted: 10 July 2026

Correspondence to:

Aditya Duta Vanda Wirakusuma

Atma Jaya Catholic University of Indonesia

dutavanda553@gmail.com

DOI: 10.52629/jamsa.v12i1.1106

Introduction

Neural Tube Defects (NTDs) occur as a result of failure in neural tube closure during embryogenesis. They are considered one of the most severe congenital disorders affecting the central nervous system. These defects arise from abnormal embryogenesis affecting the central nervous system.[1,2] In vertebrates, the central nervous system is formed through neurulation during embryogenesis, a process involving the formation of the neural tube, which later develops into the brain and spinal cord. This process occurs approximately between days 17 and 28 after fertilization.[1] The neural tube is formed when the neural plate undergoes shaping, bending, and fusion during neurulation. Closure of the neural tube occurs through fusion at the dorsal midline, resulting in the formation of a complete neural tube. Failure of this closure process may lead to neuronal degeneration and developmental abnormalities due to exposure of the neuroepithelium to the external environment. Spina bifida and anencephaly are the most common forms of NTDs.[2] Globally, Neural Tube Defects (NTDs) are among the most common congenital abnormalities, with prevalence rates ranging from 0.5 to more than 10 cases per 1,000 pregnancies, particularly in low- and middle-income countries.[1,3] Failure of neural tube closure can result in permanent neurological impairment, neuronal degeneration, motor and sensory deficits, long-term disability, and even neonatal death in severe cases, thereby contributing significantly to the morbidity and mortality associated with NTDs.[1,2,4] Neural Tube Defects (NTDs) are multifactorial disorders involving both environmental and genetic factors, including exposure to teratogenic drugs such as valproic acid.

Valproic acid (VPA) is a medication commonly used to control seizures, stabilize mood, and prevent recurrent migraine attacks. However, the use of valproic acid during pregnancy may be harmful due to its high teratogenic potential. Several studies have shown that valproic acid (VPA) is one of the antiepileptic drugs associated with the greatest teratogenic risk and is linked to various major congenital malformations in the fetus. The most commonly reported abnormalities include neural tube defects (NTDs), congenital cardiac abnormalities, skeletal malformations, and orofacial clefts. One of the mechanisms contributing to the occurrence of neural tube defects is disruption of folate metabolism. Valproic acid (VPA) may interfere with the absorption and metabolism of folic acid, and such folate disruption can inhibit neural tube closure during embryogenesis. In addition to affecting folate metabolism, valproic acid (VPA) also acts as a Histone Deacetylase Inhibitor (HDACi). HDAC inhibition may alter chromatin structure and affect gene expression during embryonic development. These alterations in gene expression can disrupt neural cell proliferation, differentiation, and development, resulting in abnormal neural tube formation and closure.[5] Valproic acid (VPA) has been reported to interfere with neural tube development during embryogenesis. However, despite the well-established association between valproic acid and neural tube defects, the underlying mechanisms responsible for VPA-induced NTDs remain incompletely understood.

Several studies have reported that embryonic exposure to valproic acid affects multiple biological processes involved in neurulation and neural tube closure. Commonly proposed mechanisms include disruption of folate metabolism, increased oxidative stress, histone deacetylase inhibition, altered DNA methylation, dysregulation of gene expression, impaired cell proliferation and differentiation, and disturbances in various embryonic signaling pathways.[5,7,8] However, most previous studies and reviews have discussed these mechanisms separately, focusing only on specific pathways. In reality, these pathways are likely interconnected and may contribute collectively to abnormalities in neural tube development. Therefore, an understanding of the integrated mechanisms involved in VPA-induced NTDs remains limited and requires a more comprehensive evaluation to clarify the interactions among the pathways underlying the pathogenesis of this condition.

Due to the limited number of studies investigating the mechanism of HDAC inhibition in valproic acid exposure, this review does not discuss this mechanism in detail. Based on this limitation, this review focuses on examining and integrating the various molecular mechanisms involved in valproic acid-induced neural tube defects. In addition, this review seeks to elucidate the relationships and interactions among the mechanistic pathways that may contribute to neurulation failure and the development of neural tube defects. By integrating these mechanisms, this review is expected to enhance current understanding, provide a more comprehensive understanding of valproic acid-induced neural tube defects, and serve as a foundation for future research development and preventive strategies.

DOI: 10.52629/jamsa.v12i1.1106

Method

Protocol and Registration

This systematic review was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020. The study protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD420261384605).

Eligibility Criteria

The literature search strategy was developed using the PICO framework (Population, Intervention, Comparison, and Outcome).

Sources and Search Strategy

The literature search was done using PubMed, ScienceDirect, Wiley, and ProQuest as databases using the keywords molecular pathogenesis, valproate, neural tube defects, apoptosis, gene expression, and folate pathway. Five authors conducted the search strategy based on the availability of each database. The retrieved studies were then selected based on the title, abstract, and full text in accordance with the eligibility criteria that had been set.

Selection Process

All records that have been found will be imported into a reference manager, and duplicates will be removed.

Table 1. PICO Framework

Population	Intervention	Comparison	Outcome
All experimental embryonic models (animal-based or cell-based), including mammalian embryos or neural stem/progenitor cell lines, exposed to valproic acid and used to model neural tube defects or related neurodevelopmental disruption (all species, all sexes).	Experimental exposure to valproic acid in embryonic models, including in vivo and in vitro systems. All dosages, timing (e.g., pre-, peri-, or post-neurulation), durations, and frequencies of exposure are eligible, provided the study evaluates molecular, epigenetic, or developmental outcomes relevant to neural tube defects.	Non-exposed controls (no valproic acid exposure) and/or exposure to alternative anti-epileptic drugs, where used as a comparator to assess molecular, epigenetic, or developmental outcomes related to neural tube defects.	Eligible outcomes include changes in apoptosis-related markers, folate-pathway alterations, oxidative stress, epigenetic changes, dysregulation of developmental signaling pathways or gene expression (e.g., Wnt, Pax3, retinoic acid signaling), and morphological or phenotypic evidence of the neural tube defects or impaired neurulation.

Study selection will be conducted independently by the review team according to the predefined eligibility criteria in two sequential phases: (1) title and abstract screening; and (2) full-text screening. Five reviewers will participate in the screening process, with each record being assessed by at least two reviewers. Discrepancies will be discussed until the conflicts have been resolved; a third reviewer will decide if an agreement cannot be achieved. During screening, exclusion criteria will be applied in the following order:

- Not an experimental embryonic model (animal or cell-based)
- No exposure to valproic acid
- No relevant outcomes related to neural tube defects or molecular/epigenetic mechanisms
- No comparator (if required for analysis)
- Non-original research (reviews, editorials, conference abstracts without sufficient data)

Data Collection Process

Data extraction will be performed independently by at least two of five reviewers using a standardized extraction form. Discrepancies will be discussed with a third reviewer until resolved. Data will be extracted from text, tables, and figures; where necessary, digital tools will be used to extract numerical data from graphs. Authors may be contacted for missing or unclear data when required. Data extraction will include study type (in vivo/in vitro), experimental design (controlled), number of groups, sample size, presence and type of comparator, and duration of study; details of valproic acid exposure, including dose/concentration, timing (e.g., gestational stage or differentiation phase), duration, frequency, and route

DOI: 10.52629/jamsa.v12i1.1106

of administration; molecular and gene expression changes relevant to neurulation (e.g., Wnt signaling, Pax3, folate-pathway genes). Data will be extracted as continuous variables (e.g., mean ± SD, fold change, relative expression levels) or equivalent reported metrics. Where necessary, values will be standardized or normalized across studies. Outcomes also include morphological or phenotypic measures of neural tube defects, such as incidence of neural tube defects (e.g., spina bifida, anencephaly), reported as dichotomous data (e.g., number of affected embryos/total). Additional outcomes may include markers of oxidative stress, apoptosis, or related developmental pathways.

Table 2. Search Strategy

Database	Search Strategy	Search Result
PubMed	("Valproic Acid"[MeSH] OR "Valproate") AND ("Histone Deacetylase Inhibitors"[MeSH] OR "HDAC inhibition") AND ("Neural Tube Defects"[MeSH] OR "Spina Bifida" OR "Anencephaly") AND ("Molecular Biology" OR "Gene Expression Regulation" OR "Epigenomics")	2
Science Direct	Valproate AND "HDAC inhibition" AND "Neural Tube Defects" AND "Molecular Mechanism" AND "Epigenetics"	14
Wiley	(Valproate OR "Valproic Acid") AND (Epigenetic* OR "Histone Acetylation" OR "Gene Expression") AND ("Neural Tube Defects" OR "Congenital Malformations" OR "Neurodevelopment")	1350
ProQuest	noft((Valproate OR "Valproic Acid")) AND noft((Epigenetic* OR "Histone Acetylation" OR "Gene Expression")) AND noft(("Neural Tube Defects" OR "Congenital Malformations" OR "Neurodevelopment"))	7

Result

Study Selection

A total of 1373 records were identified through database searching. After removing duplicates and records marked as ineligible by automation tools, 30 studies remained for title and abstract screening. A total of 23 records were excluded due to the ineligible population, intervention, comparison, and outcomes. Ultimately, 7 studies were included in this systematic review.

Study Characteristics

The seven included studies, published between 1996 and 2025, investigated the molecular mechanisms of valproic acid (VPA) induced neural tube defects (NTDs) using both in vivo and in vitro experimental models. Most studies used in vivo mouse models, particularly CD-1, SWV, and LM/Bc mice, while one study used a chicken embryo model. Two studies used in vitro stem cell models, including P19C5 stem cells and mouse embryonic stem cells. VPA exposure was generally administered during early embryonic development, the critical period for neural tube formation.

The studies mainly examined mechanisms related to apoptosis, folate metabolism, retinoic acid (RA) signaling, oxidative stress, HDAC inhibition, and developmental gene regulation. Several studies reported that VPA altered the expression of important developmental genes, including Casp3, Bax, Trp53, MTHFR, folr1, Hoxa1, and Pax3. In addition, some studies also evaluated interventions or modulators, including IFN-gamma, LE135, and N-acetylcysteine (NAC), which demonstrated potential in reducing the harmful effects of VPA exposure and lowering the incidence of neural tube defects. Involving IFN-gamma studies reported a reduction in VPA-induced NTD incidence, reflecting the

DOI: 10.52629/jamsa.v12i1.1106

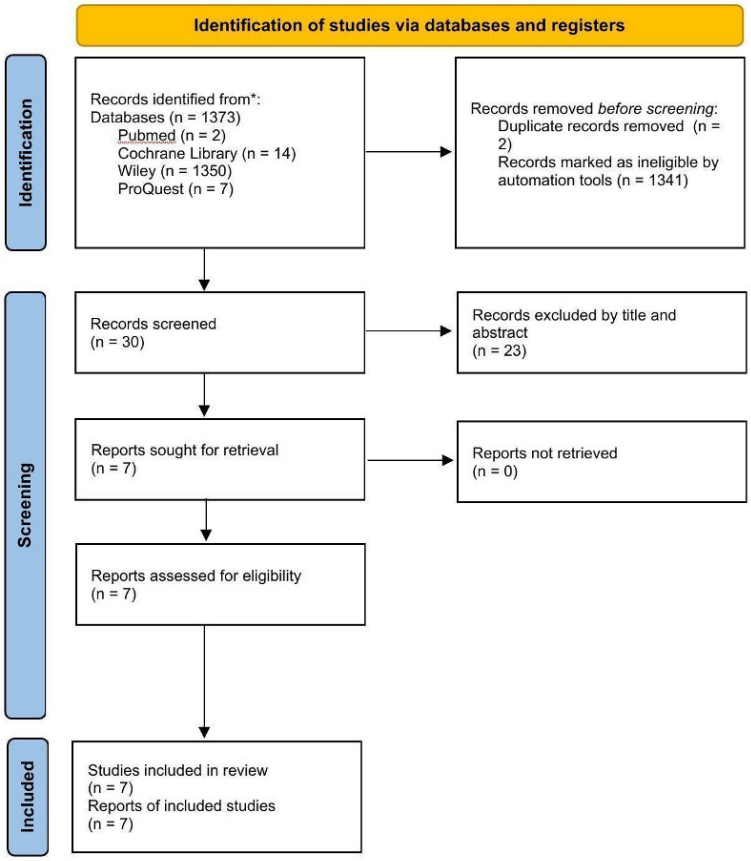

Figure 1: PRISMA Flowchart

effect of the intervention rather than VPA itself.

In addition, genetic background was found to influence the risk of VPA-induced NTDs. Studies comparing SWV and LM/Bc mice showed that the SWV strain had a higher risk of developing NTDs due to greater suppression of folate-related genes. Overall, the included studies showed that VPA disrupts multiple molecular pathways involved in neural tube development, whereas several studies evaluated interventions or modulators to reduce its teratogenic effects.

Risk of Bias Analysis

The included studies' methodological quality varies according to the SYRCLE and modified SYRCLE Risk of Bias scores.[16] Overall, the majority of studies revealed a low to moderate risk of bias in several areas, especially when it came to data completeness and outcome reporting. However, due to inadequate methodological reporting, several domains—especially those about randomization processes, allocation concealment, blinding, and variations from intended interventions—were often rated as unclear.

Many studies showed more consistent methodological quality and a comparatively decreased risk of bias, especially in areas of outcomes analysis and selective reporting. However, there was still uncertainty in areas like sequence generation, random housing, and blinding investigators or result assessors. On the contrary, multiple studies revealed greater issues across several categories, especially about experimental allocation procedures, insufficient outcome data, and a lack of transparency in the reporting of experimental methodologies.

Compared to more recent publications, older studies usually showed a higher likelihood of bias, primarily as a result of lax reporting guidelines and insufficient methodological procedure descriptions. Several "unclear" judgments in both SYRCLE and modified SYRCLE assessments were caused by common constraints found in all of the included studies, including inadequate information on allocation techniques, housing circumstances, and blinding protocols. Overall, most studies were deemed to have adequate evidence quality despite these methodological constraints; however, methodological rigor and reporting transparency still need to be improved.

Table 3. Characteristic Table

Author (Year)	Study Model	VPA Dosage & Timing	Main Molecular Target	Key Gene Alterations	Intervention/ Modulator	Main Phenotypic Outcome
Frascella et al. (2025)[9]	In vivo (CD-1 Mice)	400 mg/kg at GD 9:0	Apoptosis & Maternal Immune Stimulation (MIS)	Downregulation of Casp3, Bax, Bak1, Trp53 (after MIS)	Interferon-gamma (IFN-gamma)	Reduced NTD incidence & suppressed apoptotic genes.
Li et al. (2016)[10]	In vitro (P19C5 Stem Cells)	0.5–1.0 mM (Therapeutic range)	Retinoic Acid (RA) Signaling	Overexpression of Cdx1, Hoxa1, Cyp26a1	RA receptor antagonist (LE135)	Inhibition of EB axial elongation & patterning.
Mallela & Hrubec (2012)[11]	In vivo (CD-1 Mice)	400 mg/kg at GD 9:0	Apoptosis	Enhanced apoptotic cell population along the neural tube (TUNEL+)	IFN-gamma (Nonspecific MIS)	54% reduction in VPA-induced NTDs.
Finnell et al. (1997)[12]	In vivo (SWV vs LM/ Bc Mice)	400 mg/kg at GD 8:18	Folate Pathway & Genetic Susceptibility	Suppressed FBP-1 in sensitive strain; reduced MTHFR expression levels	Strain-dependent (Genetic background)	Higher NTD rate in SWV strain due to folate gene suppression.
Jergil et al. (2011)[13]	In vitro (Mouse Embryonic Stem Cells)	0.5 mM (Short-time exposure)	Toxicogenomic Response	Alteration in Wnt, Notch, and Hox gene clusters	VPA Analogs (Valpromide, etc.)	Prediction of developmental neurotoxicity via gene profiles.
Wlodarczyk et al. (1996)[14]	In vivo (SWV vs LM/ Bc Mice)	400 mg/kg at GD 9:0	Transcription Factors & Cell Cycle	Elevation of c-fos, c-jun, CREB and overexpression of p53 and wee-1	N/A	Accelerated developmental gene profile in sensitive strains.
Hsieh et al (2013)[15]	In vivo (chicken embryo model)	30 mM, 100 µL/egg at HH stage 22 (day 3.5)	Folate pathway, HDAC inhibition & oxidative stress	Downregulation of folr1, IGF2R, RGS2, COL6A3, EDNRB, KLF6, and Pax3	VPA as intervention, N-acetylcysteine (NAC) as modulator	Dysregulated neurulation-related gene expression associated with increased neural tube defect susceptibility.

Table 4. Risk of bias for in vivo studies (SYRCLE RoB tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Frascella et al. (2025) [9]	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Finnell et al. (1997) [12]	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Mallela & Hrubec (2012) [11]	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Wlodarczyk et al. (1996 [14]	Unclear	Yes	Unclear	Unclear	Unclear	No	Unclear	Yes	Unclear	Unclear
Hsieh et al. (2013) [15]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes

Table 4. Risk of bias for in vitro studies (Modified SYRCLE's RoB tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Jergil et al., (2011) [13]	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Unclear
Li et al., (2016) [10]	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes

DOI: 10.52629/jamsa.v12i1.1106

Discussion

This systematic review utilized seven studies, consisting of five in vivo studies and two in vitro studies. The majority of in vivo studies used a VPA dose of 400 mg/kg at GD 8.18-9.0, while in vitro studies used concentrations of 0.5–1.0 mM.

Alteration of Apoptotic Pathways and Cellular Balance

Two in vivo studies showed that exposure to VPA increased apoptosis along the neural tube. Frascella discovered that 400 mg/kg VPA treatment at gestational day 9.0 decreased apoptosis-related genes such as Casp3, Bax, Bak1, and Trp53 after maternal immunological activation with IFN-γ. This intervention was found to reduce the incidence of NTDs. Similarly, Mallela reported more TUNEL-positive cells during VPA treatment, indicating increased apoptotic cell populations along the neural tube. IFN-γ activated the mother's immune system, causing a 54% reduction in VPA-induced NTDs.

Disruption of Developmental Transcription and Folate Transport

Through an in vivo mouse model study, Wlodarczyk et al. (1996) found that VPA exposure increased the expression of transcription factor genes such as Pax-3, Emx-1, Emx-2, c-fos, c-jun, and CREB in SWV embryos. Wlodarczyk explained that Pax3 is expressed in regions of the neural tube where neural crest cells emerge and migrate, whereas c-jun and c-fos are involved in cell proliferation. In addition, bcl-2 mediates the growth-inhibitory and apoptotic effects associated with p53, while p53 plays an important role in regulating neural tube closure through cell cycle regulation. In LM/Bc embryos, VPA exposure increased the expression of bcl-2 and p53 while decreasing Pax-3 expression during neural tube closure. Furthermore, the expression ratio of bcl-2 to p53 was found to be higher for bcl-2, suggesting that VPA primarily inhibits neuroepithelial cell proliferation. These findings are important because they suggest a shift in the understanding of teratogenic mechanisms, from the assumption that teratogens induce cell death to the concept that teratogens such as VPA disrupt the balance between proliferation and differentiation. Furthermore, Hsieh et al. (2013) expanded these findings by identifying 21 genes disrupted by VPA, including five major interacting genes potentially associated with neural tube defects (NTDs), namely IGF2R, RGS4, COL6A3, EDNRB, and KLF6. Gene ontology analysis showed that the affected genes are involved in translation, signal transduction, transcription, cell adhesion, neural cell migration, transport, and organismal development. Additionally, expression of the folate receptor gene (folr1) by VPA was reported, suggesting a molecular link between VPA exposure and folate deficiency. Taken together, the findings of Wlodarczyk et al. and Hsieh et al. suggest that VPA disrupts neurulation through alterations in developmental transcription factors and folate-related pathways.[17] These molecular disturbances impair essential cellular processes required for neural tube formation. In addition to these mechanisms, accumulating evidence suggests that disruption of retinoic acid (RA) signaling represents another important pathway contributing to VPA-induced teratogenicity.

Retinoic Acid Signaling and Developmental Gene Dysregulation

In an in vitro study using P19C5 stem cells, Li reported that therapeutic VPA doses (0.5-1.0 mM) altered retinoic acid (RA) signaling pathways, which resulted in upregulation of developmental genes such as Cdx1, Hoxa1, and Cyp26a1. Furthermore, treatment with the RA receptor antagonist LE135 reduced embryoid body axial elongation and patterning, indicating that abnormal RA signaling contributes to poor embryonic development. Using a P19C5 embryoid body (EB) model, Li stated how EBs that were not exposed to VPA exhibited normal axial elongation, whereas treatment with 0.6 mM VPA inhibited elongation. RNA levels of RA target genes and the trend of increased Cdx1 expression from 0.5 to 1.8, and Hoxa1 expression from 0.4 to 1.4, on the first day of treatment were spotted. Cdx1, Hoxa1, and Cyp26a1 are RA target genes that collectively function to maintain retinoic acid signaling homeostasis during gastrulation and neurulation. However, when assessed using the RARE-Luc reporter assay, RA levels did not increase, indicating that VPA does not elevate RA concentrations. Therefore, the increased expression of Hoxa1 and Cdx1 was not caused by elevated RA levels, but rather by histone hyperacetylation, which renders chromatin more responsive to RA signaling. Consistent with these findings, VPA exposure has been shown to functionally mimic retinoic acid excess by aberrantly activating RA signaling pathways.[18] This dysregulation promotes the overexpression of key caudalizing genes, including

DOI: 10.52629/jamsa.v12i1.1106

Cdx1 and Hoxa1, which are essential for determining positional identity along the embryonic axis.[19]

Mechanistically, Jergil et al. (2011) showed that VPA induces rapid toxicogenomic shifts in Hox, Wnt, and Notch gene clusters within hours of exposure. This suggests that VPA interferes with the "epigenetic clock" of the embryo. By causing a premature or exaggerated expression of these genes, VPA disrupts the precisely timed coordination of cell migration and differentiation required for neural fold elevation. This "molecular chaos" prevents the neural folds from meeting at the midline, leading to the physical manifestation of neural tube defects (NTDs) and associated axial skeletal malformations.[20] Taken together, the findings of Li et al. and Jergil et al. suggest that VPA disrupts embryonic development through coordinated alterations in retinoic acid (RA) signaling and epigenetic regulation. These molecular disturbances impair the tightly regulated processes of neural patterning, cell differentiation, and neural tube closure, thereby contributing to the development of neural tube defects (NTDs). Although previous studies have demonstrated that VPA disrupts multiple molecular pathways involved in neurulation, not all fetuses exposed to VPA develop neural tube defects (NTDs). This observation suggests that the embryonic response to VPA exposure is influenced not only by the molecular effects of VPA itself but also by interindividual differences in genetic susceptibility.

Genetic Susceptibility: Determinants of Embryonic Resilience

Finnell et al. (1997) demonstrated this by comparing two mouse strains administered the same dose of VPA and found that the SWV strain was highly susceptible to NTDs, whereas the LM/Bc strain was more resistant. In the susceptible SWV strain, VPA reduced the expression of the FBP-1 (folate-binding protein-1) gene and did not alter MTHFR expression, resulting in folate deficiency. In contrast, in the resistant LM/Bc strain, VPA increased the expression of both FBP-1 and MTHFR genes, which may protect against the teratogenic effects of VPA. Findings from Finnell suggest that individuals who can upregulate folate pathway gene expression in response to VPA exposure may be protected from NTDs. Similarly, Wlodarczyk et al. (1996) reported that in the susceptible SWV strain, VPA decreased the expression of the bcl-2 and p53 genes, leaving neuroepithelial cells without adequate protection against the teratogenic effects of VPA. In contrast, in the resistant LM/Bc strain, VPA increased the expression of bcl-2 and p53. These findings indicate that individuals capable of increasing bcl-2 expression in response to the teratogenic effects of VPA may exhibit greater resistance to NTDs. Taken together, the findings of Finnell et al. and Wlodarczyk et al. suggest that the teratogenic effects of VPA are influenced not only by specific molecular pathways but also by the embryo's genetic capacity to activate compensatory responses. Therefore, genetic susceptibility appears to be a key determinant of embryonic resilience against VPA-induced neural tube defects (NTDs). This genetic resilience aligns with the insights from Mallela and Hrubec (2012) regarding the reduction of VPA-induced NTDs. Rather than suggesting that VPA itself decreases the defect risk, their study clarifies that the reduction is strictly driven by maternal immune stimulation, which effectively mitigates VPA-induced teratogenicity by normalizing dysregulated cellular apoptosis and preventing malformations. A more comprehensive analysis of this maternal immune modulation is provided in the following section.

The One-Carbon Metabolism: MTHFR and FBP-1 qs Protective Buffer

The genes MTHFR (5,10-methylenetetrahydrofolate reductase) and FBP-1 (Folate Binding Protein-1) are central to this protective mechanism. In the highly sensitive SWV strain, VPA insult caused a significant suppression of MTHFR and FBP-1 mRNA expression, effectively "strangling" the embryo's ability to process folate.[21] This metabolic failure leads to localized folate deficiency at the neural folds, even if maternal folate levels are normal. In contrast, resistant strains maintain stable or up-regulated expression of these genes, allowing them to maintain the DNA synthesis and methylation reactions necessary for neural tube closure. This underscores that VPA teratogenicity is not a "one-size-fits-all" phenomenon but is dictated by a complex interaction between the drug and the host's metabolic genetic blueprint malformations.[17]

One of the most novel findings of this review comes from the study by Frascella et al. (2025), which explored the role of maternal immune stimulation (MIS) in modulating teratogenic effects caused by VPA. This study suggests that modulation of MIS may partially protect embryos from valproate-induced NTDs. This study showed suppressed apoptotic genes through the downregulation of Casp3, Bax, Bak1, Trp53, and lower NTD incidence after stimulation with interferon-gamma (IFN-γ), with the VPA group having 45.3%

DOI: 10.52629/jamsa.v12i1.1106

incidence of NTDs, and the IFN-γ group at 21.2%. These significant changes suggest that MIS may influence embryonic resilience against VPA-induced teratogenicity, particularly through the apoptotic pathway. Mechanistically, MIS may modulate these effects by altering the inflammatory signaling, oxidative stress responses, or modulating downstream apoptotic cascades. However, these findings indicate that a reduction in NTD incidence was observed only when maternal immune stimulation (MIS) with interferon-gamma (IFN-γ) was administered concomitantly with VPA exposure. Therefore, the observed protective effect is more likely attributable to the ability of MIS to modulate apoptotic pathways. In contrast, VPA itself remains a well-established teratogen associated with an increased risk of neural tube defects (NTDs). This combined perspective demonstrates that the pathological cascade driven by VPA-induced folate disruption and oxidative stress ultimately converges into widespread embryonic apoptosis, a mechanism that is directly countered by the protective pathways activated during maternal immune stimulation.[23]

Clinical Implications

Although current clinical guidelines recommend avoiding valproate during pregnancy because of its well-established teratogenicity, its clinical use is strictly contraindicated during pregnancy due to its well-established, severe teratogenic risks. Avoiding valproate use for childbearing patients is heavily mandated, especially whenever effective alternatives such as lamotrigine or levetiracetam are available to minimize the risk of fetal malformations while maintaining maternal seizure control. Consequently, a small subset of women whose seizures cannot be adequately controlled with alternative antiseizure medications may still require continued valproate therapy after careful individualized risk-benefit assessment.[22] This presents a major clinical dilemma for women of childbearing age, where discontinuing valproate may endanger both the mother and the fetus through uncontrollable seizures, while continuation increases teratogenic risks.[5] The present review suggests that VPA-induced neural tube defects arise through interconnected mechanisms involving apoptosis, folate-pathway disruption, oxidative stress, retinoic acid signaling, and dysregulation of developmental gene expression. Therefore, MIS-related findings may open future opportunities for preventive therapeutic strategies by demonstrating that maternal immune stimulation may attenuate apoptotic gene expression and reduce neural tube defect incidence in experimental models. Although this strategy is still highly experimental and cannot be applied clinically at present, modulation of the maternal immune pathway can be considered as an adjunctive protective approach for pregnant patients with generalized epilepsy. Further mechanistic studies are necessary before clinical application can be considered.

Strengths and Limitations

This review integrates multiple domains involved in valproate-induced NTD pathogenesis, including apoptosis, folate-pathway dysregulation, oxidative stress, epigenetic alteration, and developmental signaling abnormalities. This provides broad molecular synthesis across diverse experimental systems. The inclusion of both in vivo and in vitro embryonic models provides a comparison of molecular responses across species. This review also highlights potential therapeutic strategies for generalized epilepsy, such as maternal immune stimulation, which is currently still underexplored in current literature. However, limitations in this review should be acknowledged. Heterogeneity was still present among included studies regarding animal species, developmental stage, valproate dosage, timing of exposure, and outcome measurements. These limitations restricted direct comparisons between studies.

Further Directions

The findings of this review reinforce that valproic acid-associated teratogenicity is multifactorial, involving effects such as apoptosis, folate dysregulation, oxidative stress, and altered developmental gene signaling. An understanding of these effects may contribute to more thorough planning of preventive strategies in pregnant patients requiring antiseizure medication. Caution regarding valproate use during pregnancy is still needed, particularly during early neurulation. However, because some patients with generalized epilepsy cannot achieve adequate seizure control with alternative antiseizure medications, valproate may remain necessary in selected cases despite its teratogenic risk. Therefore, identifying protective interventions is needed. Future studies should focus on therapeutic strategies capable of reducing teratogenic risk while still controlling seizures effectively. In particular, the emerging evidence surrounding MIS shows potential for further investigation as a possible therapeutic target. Standardized exposure controls

DOI: 10.52629/jamsa.v12i1.1106

and approaches also need to be considered to improve mechanistic understanding and to support the development of safer therapeutic strategies for pregnant women who require valproate.

Conclusion

This systematic review shows that neural tube defects (NTDs) triggered by valproic acid (VPA) are mediated by several interrelated mechanisms, including VPA-induced oxidative stress, apoptosis, folate-pathway dysregulation, epigenetic modification, and disruption of developmental signaling pathways such as retinoic acid signaling. Studies conducted in vitro and in vivo consistently show that VPA modifies the expression of genes necessary for cellular homeostasis, neurulation, and embryonic patterning.

The results also imply that teratogenic outcomes are influenced by genetic vulnerability, specifically through variations in folate metabolism and anti-apoptotic gene responses. Although this method is still experimental, recent studies on maternal immune stimulation (MIS) suggest a possible protective function against VPA-induced teratogenicity.

Overall, it seems that host susceptibility factors and molecular signaling disturbance interact intricately to cause VPA teratogenicity. To support the development of safer therapeutic and preventive approaches for pregnant patients in need of VPA treatment, more mechanistic and standardized experimental research is required.

Acknowledgement

The authors declare that no declarations are relevant to this systematic review.

Author's Contribution

All authors contributed equally to this review. The tasks were fairly distributed, including literature screening, data analysis, and manuscript drafting. Each author was responsible for specific parts of the writing and revision process. All authors read and approved the final manuscript.

References

1. Avagliano L, Massa V, George TM, Qureshy S, Bulfamante GP, Finnell RH. Overview on neural tube defects: From development to physical characteristics. Birth Defects Res. 2019;111(19):1455-67. doi:10.1002/bdr2.1380.
2. Greene NDE, Copp AJ. Neural tube defects. Annu Rev Neurosci. 2014;37:221-42. doi:10.1146/annurev-neuro-062012-170354.
3. Zaganjor I, Sekkarie A, Tsang BL, Williams J, Razzaghi H, Mulinare J, et al. Describing the prevalence of neural tube defects worldwide: A systematic literature review. PLoS One. 2016;11(4):e0151586. doi:10.1371/journal.pone.0151586.
4. Kondo A, Matsuo T, Morota N, Kondo AS, Okai I, Fukuda H. Neural tube defects: Risk factors and preventive measures. Congenit Anom (Kyoto). 2017;57(5):150-6. doi:10.1111/cga.12227.
5. Ornoy A, Echefu B, Becker M. Valproic acid in pregnancy revisited: Neurobehavioral, biochemical and molecular changes affecting the embryo and fetus in humans and in animals: A narrative review. Int J Mol Sci. 2024;25(1):390. doi:10.3390/ijms25010390.
6. Perucca E. Pharmacological and therapeutic properties of valproate: A summary after 35 years of clinical experience. CNS Drugs. 2002;16(10):695-714. doi:10.2165/00023210-200216100-00004.
7. Fathe K, Palacios A, Finnell RH. Brief report: Novel mechanism for valproate-induced teratogenicity. Birth Defects Res A Clin Mol Teratol. 2014;100(8):592-7. doi:10.1002/bdra.23277.
8. Akimova D, Wlodarczyk BJ, Lin Y, Ross ME, Finnell RH, Chen Q, et al. Metabolite profiling of whole murine embryos reveals metabolic perturbations associated with maternal valproate-induced neural tube closure defects. Birth Defects Res. 2017;109(2):106-19. doi:10.1002/bdra.23583.
9. Frascella D, Mallela MK, Cullen R, Hrubec TC. Maternal immune stimulation with interferon-gamma limits embryonic valproic acid induced apoptotic gene expression. Birth Defects Res. 2025;117(11):e2541. doi:10.1002/bdr2.2541.
10. Li ASW, Marikawa Y. Adverse effect of valproic acid on an in vitro gastrulation model entails activation of retinoic acid signaling. Reprod Toxicol. 2016;66:68-83. doi:10.1016/j.reprotox.2016.09.015.
11. Mallela M, Hrubec T. Reduction in valproic acid-induced neural tube defects by maternal immune stimulation: role of apoptosis. Birth Defects Res B Dev Reprod Toxicol. 2012;95(4):296-303. doi:10.1002/bdrb.21018.
12. Finnell RH, Wlodarczyk BC, Craig JC, Piedrahita JA, Bennett GD. Strain-dependent alterations in the expression of folate pathway genes following teratogenic exposure to valproic acid in a mouse model. Am J Med Genet. 1997;70(3):303-11. doi:10.1002/(SICI)1096-8628(19970613)70:3<303::AID-AJMG17>3.0.CO;2-P.
13. Jergil M, Forsberg M, Salter H, Stockling K, Gustafson AL, Dencker L, et al. Short-time gene expression response to valproic acid and valproic acid analogs in mouse embryonic stem cells. Toxicol Sci. 2011;121(2):328-42. doi:10.1093/toxsci/kfr070.
14. Wlodarczyk BC, Craig JC, Bennett GD, Calvin JA, Finnell RH. Valproic acid-induced changes in gene expression during neurulation in a mouse model. Teratology. 1996;54(6):284-97. doi:10.1002/(SICI)1096-9926(199612)54:6<284::AID-TERA3>3.0.CO;2-Z.
15. Hsieh CL, Chen KC, Ding CY, Tsai WJ, Wu JF, Peng CC. Valproic acid substantially downregulated genes folr1, IGF2R, RGS2, COL6A3, EDNRB, KLF6, and pax-3, N-acetylcysteine alleviated most of the induced gene alterations in chicken embryo model. Rom J Morphol Embryol. 2013;54(4):993-1004.
16. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14:43.

doi:10.1186/1471-2288-14-43.

17. Reynolds EH, Green R. Valproate and folate: Congenital and developmental risks. Epilepsy Behav. 2020;108:107068. doi:10.1016/j.yebeh.2020.107068. PMID: 32375098.
18. Wu D, Faheem Ahmed Khan, Zhang K, Nuruliarizki Shinta Pandupuspitasari, Windu Negara, Guan K, et al. Retinoic acid signaling in development and differentiation commitment and its regulatory topology. Chemico-Biological Interactions. 2024 Jan 1;387:110773–3.
19. Afzal Z, Lange JJ, Nolte C, McKinney S, Wood C, Paulson A, et al. Shared retinoic acid responsive enhancers coordinately regulate nascent transcription of Hoxb coding and non-coding RNAs in the developing mouse neural tube. Development. 2023 May 15;150(10).
20. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Comparative risk of major congenital malformations with eight different antiepileptic drugs: a prospective cohort study of the EURAP registry. The Lancet Neurology. 2018 Jun;17(6):530–8.
21. Xie X, Li C, Yu J, Chang S, Cheng X, Wang F, et al. MTHFD1 is critical for the negative regulation of retinoic acid receptor signalling in anencephaly. Brain. 2023 Mar 14;146(8):3455–69.
22. World Health Organization. mhGAP-Humanitarian Intervention Guide (mhGAP-HIG): Addendum: Use of valproic acid (sodium valproate) in women and girls of childbearing potential [Internet]. Geneva: World Health Organization; 2023. Available from: https://cdn.who.int/media/docs/default-source/brain-health/mhgap_hig_v4_0(08032022).pdf
23. Massaad J, Baydoun S, El-Hajj M, Kobeissy F, Sabra R. Valproic Acid in Pregnancy Revisited: Neurobehavioral, Biochemical and Molecular Changes Affecting the Embryo and Fetus in Humans and in Animals. Biomedicines. 2024;12(1):155. doi:10.3390/biomedicines12010155.

Volume 12 | 2026 Release

Journal of Asian Medical Students' Association

www.jamsa.amsa-international.org