Therapeutic Efficacy of Plant-Derived α-Linolenic Acid in a DMBA-Induced Mammary Carcinoma Model: A Sustainable Alternative to Marine Omega-3s

Anurag Kumar[1], Gaurav Kaithwas[1*]

1. Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow- 226 025, Uttar Pradesh, India.

***Corresponding Author**
Dr. Gaurav Kaithwas
Department of Pharmaceutical Sciences
School of Pharmaceutical Sciences
Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Raebareli Road
Lucknow- 226 025 (Uttar Pradesh), India
Email: gauravpharm@gmail.com
Ph: +91-9670204349

Abstract

Omega-3 polyunsaturated fatty acids (PUFAs), particularly α-linolenic acid (ALA), are recognized for their crucial role in modulating inflammation, supporting cardiovascular health, and preventing chronic diseases. While marine-derived omega-3 fatty acids have been extensively studied, issues related to sustainability, environmental contaminants, and oxidative instability have led to increased interest in plant-based alternatives. This study investigates the therapeutic potential of purified plant-derived ALA (P-ALA) compared to marine-derived ALA (M-ALA) in a 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary gland carcinoma model in female albino Wistar rats. The effects on body weight variation and lipid profile were evaluated to assess systemic health and metabolic function. Results demonstrated that P-ALA effectively mitigated weight loss and improved lipid parameters, showing superior efficacy at higher doses compared to M-ALA. These findings highlight the potential of plant-based ALA as a sustainable, bioactive alternative for developing safe, vegetarian-friendly therapeutic supplements targeting cancer-related metabolic dysfunction.

Keywords: - α-Linolenic acid (ALA), omega-3 fatty acids, plant-derived PUFAs, mammary gland carcinoma, flaxseed.

1. Introduction

Polyunsaturated fatty acids (PUFAs), particularly omega-3 (ω-3) fatty acids such as α-linolenic acid (ALA), are essential components of human nutrition due to their wide-ranging physiological benefits, including anti-inflammatory, cardioprotective, and neuroprotective effects. Unlike saturated and monounsaturated fatty acids, PUFAs cannot be synthesized endogenously and must be obtained from dietary sources. Marine-derived ω-3 PUFAs like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been widely studied for their therapeutic potential; however, concerns related to environmental sustainability, heavy metal contamination, oxidative instability, and undesirable sensory attributes (e.g., fishy odor) have spurred growing interest in plant-based alternatives (1,2).

Flaxseed (*Linum usitatissimum*) has emerged as a promising plant-derived source of ALA, which serves as a metabolic precursor for EPA and DHA, and plays a critical role in vegetarian and vegan diets (3,4). In addition to its high ALA content, flaxseed offers further nutritional advantages, including dietary fiber, lignans, and antioxidants. Recent evidence supports the therapeutic potential of ALA in regulating inflammation, preventing carcinogenesis, and improving cardiovascular health, making it a viable candidate for dietary interventions and functional food development (5–7).

This study aims to systematically evaluate the efficacy of purified plant-derived ALA (P-ALA) in comparison with marine-derived ALA (M-ALA) in a 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary gland carcinoma model in female albino Wistar rats. Particular emphasis is placed on body weight variation and lipid profiling, as these parameters serve as

critical indicators of systemic toxicity, metabolic function, and therapeutic response. Body weight reflects the overall health status of the organism and is sensitive to both disease progression and recovery. Meanwhile, lipid profile analysis provides insight into lipid metabolism, oxidative stress, and the efficacy of intervention strategies (8,9).

By assessing the impact of P-ALA on these physiological markers, the study aims to establish its potential as a sustainable, cost-effective, and vegetarian-friendly alternative to marine-based ω-3 fatty acids. Furthermore, by addressing the limitations associated with marine-derived supplements, this research contributes to the development of safer, more accessible, and environmentally conscious therapeutic options for managing cancer-associated metabolic dysfunction (10–12).

2. Materials and Methods

2.1 Materials

P-ALA was extracted from *Linum usitatissimum* using the methodology previously published(13). The commercially available M-ALA served as the comparative standard. Tamoxifen citrate was used as a positive control.

2.2. Methods

2.2.1. Experimental Animals: - Female *albino wistar* rats of 100 ±20 g body weight was procured from the Central Animal House Facility, Babasaheb Bhimrao Ambedkar University, Lucknow. The animals were housed in polypropylene cages under controlled conditions (25 ± 1°C, 12 h light/dark cycle), with a free access to a standard pellet diet and drinking water. The experiment was performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animals and ethics, Department of animal welfare, Government of India.

2.2.2. Experimental design: - The animals were divided into seven groups (n = 6 per group) for the treatment protocol. Group I served as the normal control and received no any treatment. Group II obliged as the toxic control was administered DMBA intravenously at a dose of 8 mg/kg to induce mammary gland carcinoma. Group III received TAM orally at 1 mg/kg/day in combination with DMBA (8 mg/kg, i.v.). Groups IV and V were treated with P-ALA at doses of 0.25 ml/kg and 0.5 ml/kg orally, respectively, along with DMBA (8 mg/kg, i.v.). Groups VI and VII received a M-ALA at the same respective doses of 0.25 ml/kg and 0.5 ml/kg orally, each co-administered with DMBA (8 mg/kg, i.v.). This design allowed for a comparative evaluation of plant-derived versus marketed ALA formulations at varying doses in the context of DMBA-induced mammary gland carcinoma.

2.2.3. Weight variation: - Body weight was recorded using a digital precision balance with ±0.01 g accuracy to monitor the physiological status of the animals throughout the study. Initial body weights were measured prior to the start of treatment, followed by regular interval

measurements (e.g., weekly) until the completion of the experimental period. Final body weights were documented on the last day of treatment. To ensure consistency and minimize variability due to recent food or water intake, all animals were fasted overnight prior to each weighing session(14).

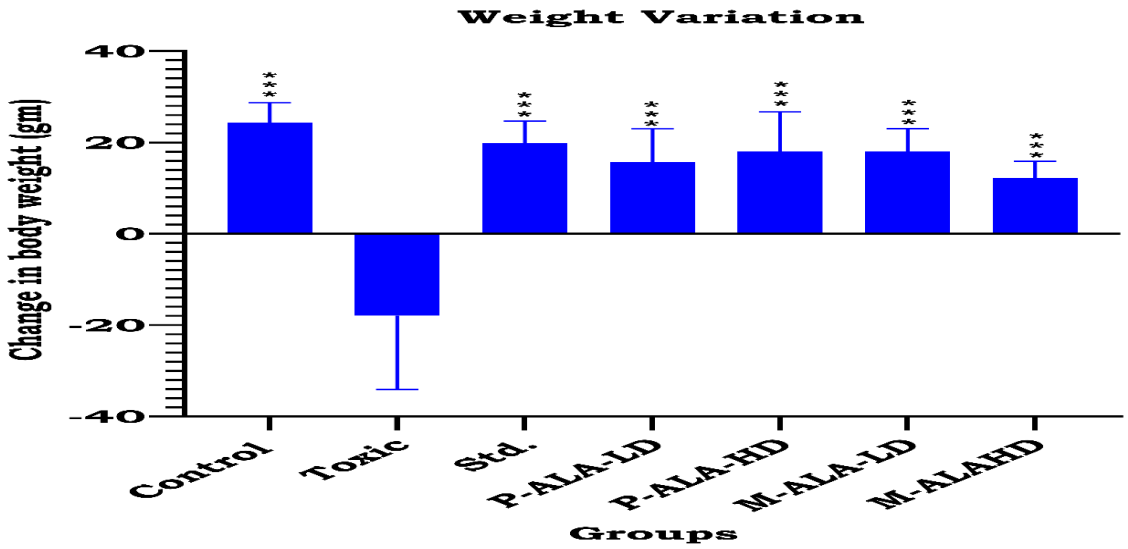
2.2.4. Biochemical Evaluation of Serum Lipid Parameters

Whole blood was centrifuged at 1250g for 15 min to obtain serum. Serum were used for measurement of total cholesterol, high density lipoprotein cholesterol (HDL), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL) and fatty acid composition of phospholipids (15).

3. Results and Discussion

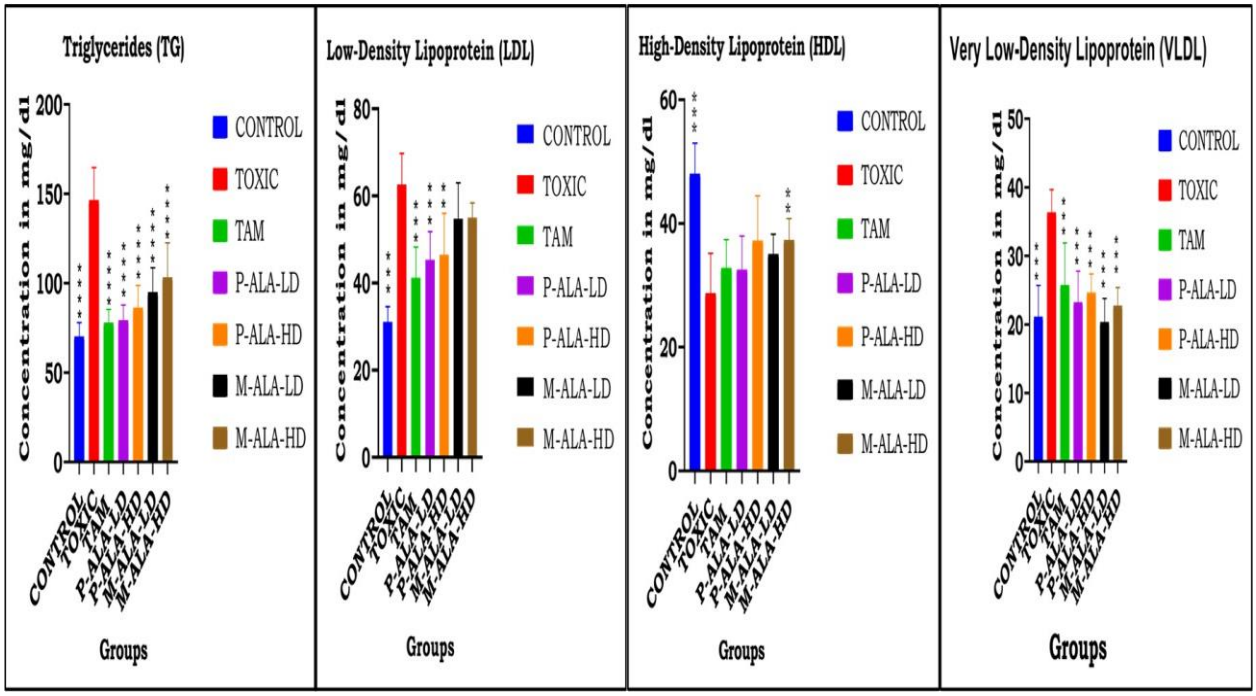
The column graph illustrates the variation in body weight across different experimental groups over the treatment period. Animals in the control group showed a consistent increase in body weight, whereas the toxic group exhibited a significant reduction. The standard group demonstrated a recovery in body weight relative to the toxic group. Treatment with M-ALA-LD resulted in moderate weight recovery, while M-ALA-HD showed a marked recovery**.** P-ALA-LD exhibited moderate improvement, whereas P-ALA-HD demonstrated the most significant recovery, with levels approaching those of the control group figure 1.Variation in Animal Weight in DMBA-Induced Albino Wistar Rat Models. The column graph illustrates the variation in body weight of animals throughout the study period. The average body weight of each animal group was measured from the first to the last day of treatment. Data are expressed as mean ± SD (n = 6)**.** Statistical analysis was performed using two-way ANOVA followed by Bonferroni's post-hoc test**.**

Figure 1: Effect of α-Linolenic Acid on Lipid profile in a DMBA-Induced Mammary Carcinoma Model



Significance was determined at ****$p \leq 0.0001$ when compared to the toxic group under the same treatment conditions. Body weight is a critical indicator of overall health, reflecting the interplay of metabolism, nutritional intake, and systemic toxicity. In this study, significant variations in body weight were observed among the experimental groups due to toxic exposure and subsequent treatment interventions. The toxic group exhibited substantial weight loss, highlighting severe systemic toxicity that likely impaired metabolic processes, appetite, and overall physiological function, emphasizing the detrimental impact of the toxin on the animals' health.

Figure 2: Effect of α-Linolenic Acid on Lipid profile in a DMBA-Induced Mammary Carcinoma Model



Both standard treatment and ALA supplementation, in both marketed and purified forms, demonstrated protective effects as evidenced by improved body weights. The extent of recovery was dose-dependent, with M-ALA-HD and P-ALA-HD being more effective than their low-dose counterparts M-ALA-LD and P-ALA-LD**.** Notably, the high-dose purified ALA group (P-ALA-HD) showed the most significant recovery in body weight, surpassing even the marketed ALA high-dose group. This suggests that the purified form of ALA has greater bioavailability or potency, enhancing its effectiveness in mitigating toxic effects.This recovery in body weight following ALA supplementation is likely attributed to its antioxidant and anti-inflammatory properties, which mitigate oxidative stress and cellular damage caused by the toxin, thereby restoring metabolic functions and promoting normal growth (16,17).The bar graph illustrating lipid profile analysis shows the concentrations of triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) across various experimental groups figure 2. Lipid Profile Analysis (TG, LDL, HDL, VLDL) Across Different Treatment Groups. The bar graph shows the concentrations of TG, LDL, HDL, and VLDL in various groups. Data are presented as mean ± SD (n = 6)**.** Statistical

analysis was conducted using one-way ANOVA followed by Bonferroni's post-hoc test, with significance set at $P \leq 0.001$ compared to the toxic group under similar treatment conditions. Lipid profile parameters are critical indicators of metabolic health and cardiovascular risk. The toxic exposure in this study caused profound dysregulation of lipid metabolism, characterized by increased TG, LDL, and VLDL levels and decreased HDL levels**.** These alterations signify enhanced lipid peroxidation, impaired lipoprotein transport, and reduced antioxidant defenses**,** all of which are hallmarks of toxicity-induced metabolic disruption. Both standard treatment and ALA supplementation exhibited dose-dependent restoration of lipid parameters, with M-ALA-HD and P-ALA-HD being more effective than their low-dose counterparts. Notably**,** P-ALA-HD showed superior efficacy**,** suggesting that purified ALA might possess better bioavailability or potency**,** thereby enhancing its lipid-regulating effects. The improvements in lipid profiles can be attributed to ALA's antioxidant and anti-inflammatory properties**,** which mitigate oxidative stress and inflammation, helping to restore lipid homeostasis (14,16,17).

4. Conclusion

The study demonstrates that purified plant-derived α-linolenic acid (P-ALA), particularly at high doses, offers significant therapeutic potential in mitigating the adverse effects of DMBA-induced mammary gland carcinoma in albino Wistar rats. ALA supplementation, in both marketed and purified forms, showed dose-dependent improvements in body weight and lipid profile, two critical indicators of systemic health and metabolic function. The high-dose P-ALA group (P-ALA-HD) was especially effective, showing near-complete recovery of body weight and significant normalization of lipid parameters—surpassing even the efficacy of marine-derived ALA (M-ALA). These results suggest that purified plant-based ALA not only possesses superior bioavailability and potency but also offers a sustainable, vegetarian-friendly alternative to marine-derived omega-3 fatty acids. Its antioxidant and anti-inflammatory properties are likely responsible for the observed protective effects, underscoring its potential role in the development of novel dietary supplements or adjunct therapies for managing cancer-related metabolic dysfunction.

5. Acknowledgement

The work presented in this manuscript was fully supported by an extramural grant from the Ministry of Food Processing Industries, Government of India (Grant No. Q-11/32/2018-R&D), New Delhi.

6. Reference

1. Saini RK, Prasad P, Sreedhar RV, Akhilender Naidu K, Shang X, Keum YS. Omega-3 Polyunsaturated Fatty Acids (PUFAs): Emerging Plant and Microbial Sources, Oxidative Stability, Bioavailability, and Health Benefits-A Review. Antioxidants (Basel, Switzerland). 2021 Oct;10(10).
2. Shahidi F, Ambigaipalan P. Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. Annu Rev Food Sci Technol. 2018 Mar;9:345–81.

3. Nowak W, Jeziorek M. The Role of Flaxseed in Improving Human Health. Healthc (Basel, Switzerland). 2023 Jan;11(3).
4. Parikh M, Maddaford TG, Austria JA, Aliani M, Netticadan T, Pierce GN. Dietary Flaxseed as a Strategy for Improving Human Health. Nutrients. 2019 May;11(5).
5. Mueed A, Shibli S, Korma SA, Madjirebaye P, Esatbeyoglu T, Deng Z. Flaxseed Bioactive Compounds: Chemical Composition, Functional Properties, Food Applications and Health Benefits-Related Gut Microbes. Foods (Basel, Switzerland). 2022 Oct;11(20).
6. Rizzo G, Baroni L, Lombardo M. Promising Sources of Plant-Derived Polyunsaturated Fatty Acids: A Narrative Review. Int J Environ Res Public Health. 2023 Jan;20(3).
7. Al-Madhagy S, Ashmawy NS, Mamdouh A, Eldahshan OA, Farag MA. A comprehensive review of the health benefits of flaxseed oil in relation to its chemical composition and comparison with other omega-3-rich oils. Eur J Med Res. 2023 Jul;28(1):240.
8. Dalimunthe A, Carensia Gunawan M, Dhiya Utari Z, Dinata MR, Halim P, Estherina S Pakpahan N, et al. In-depth analysis of lupeol: delving into the diverse pharmacological profile. Front Pharmacol. 2024;15:1461478.
9. Jeganathan A, Arunachalam K, Byju A, Rani George A, Sajeev S, Thangasamy K, et al. Chitosan Nanoparticle-Mediated Delivery of Alstonia venenata R.Br. Root Methanolic Extract: A Promising Strategy for Breast Cancer Therapy in DMBA-Induced Breast Cancer in Sprague Dawley Rats. Antioxidants [Internet]. 2024;13(12). Available from: https://www.mdpi.com/2076-3921/13/12/1513
10. Ayub A, Rahayu F, Khamidah A, Antarlina SS, Iswari K, Supriyadi K, et al. Harnessing microalgae as a bioresource for nutraceuticals: advancing bioactive compound exploration and shaping the future of health and functional food innovation. Discov Appl Sci [Internet]. 2025;7(5):389. Available from: https://doi.org/10.1007/s42452-025-06916-3
11. Qin J, Kurt E, LBassi T, Sa L, Xie D. Biotechnological production of omega-3 fatty acids: current status and future perspectives. Front Microbiol. 2023;14:1280296.
12. Mahmud N, Valizadeh S, Oyom W, Tahergorabi R. Exploring functional plant-based seafood: Ingredients and health implications. Trends Food Sci Technol [Internet]. 2024;144:104346. Available from: https://www.sciencedirect.com/science/article/pii/S0924224424000220
13. Kumar A, Yadav RK, Kumar Shrivastava N, Kumar R, Kumar D, Singh J, et al. Optimization of novel method for isolation of high purity food grade α-linolenic acid from Linum usitatissimum seeds. LWT [Internet]. 2023;189:115466. Available from: https://www.sciencedirect.com/science/article/pii/S0023643823010459
14. Feige-Diller J, Krakenberg V, Bierbaum L, Seifert L, Palme R, Kaiser S, et al. The Effects of Different Feeding Routines on Welfare in Laboratory Mice. Front Vet Sci. 2019;6:479.
15. Souza LA de, Hunka MM, Rezende Júnior SF de, Silva CJFL da, Manso HEC da CC, Simões J, et al. Potential use of beet-pulp concentrate supplementation in athletic horse. Heliyon. 2025 Jan;11(1):e40961.
16. Vajdi M, Abbasalizad Farhangi M. Alpha-lipoic acid supplementation significantly

reduces the risk of obesity in an updated systematic review and dose response meta-analysis of randomised placebo-controlled clinical trials. Int J Clin Pract. 2020 Jun;74(6):e13493.

17. Kucukgoncu S, Zhou E, Lucas KB, Tek C. Alpha-lipoic acid (ALA) as a supplementation for weight loss: results from a meta-analysis of randomized controlled trials. Obes Rev an Off J Int Assoc Study Obes. 2017 May;18(5):594–601.