Okra-derived Dietary Carotenoid Lutein against Breast Cancer, with an Approach towards Developing a Nutraceutical Product: A Meta-analysis Study

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Objective:** Cancer chemoprevention with phytochemicals such as “lutein” derived from the vegetable okra could prove beneficial. Therefore, the objective of this study was to perform a meta-analysis of “lutein” against the breast cancer cell lines (MCF-7) and to establish the possible development of lutein based nutraceuticals.

**Methodology:** A literature survey was performed using online data bases such as PubMed, Google scholar, and EMBASE, from 2000 to 2020 by using keywords such as “Lutein”, “Anticancer
1. INTRODUCTION

Cancer ranks as one of the major causes of death in humans and prevents increasing life expectancy prevents increasing life expectancy around the world [1]. According to Global Cancer Statistics (GLOBOCAN 2020) report, approximately 19.3 million new cancer cases as well as around 10 million cancer deaths has been reported globally in the year 2020 [1,2]. Among the various types of cancers, female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million cases (11.7%), followed by lung (11.4%), colorectal, prostate (7.3%), and stomach cancers. Lung cancer continued to be the predominant cause of cancer death, with an estimated 1.8 million deaths, followed by colorectal, liver, stomach, and female breast cancers [3,4]. Breast cancer is one of the most prevalent forms of malignant cancers and mortality rate in breast cancers are high in developed countries and there is also a rapid increase in the number of cancer cases in developing countries [5]. This suggests that there is a continuous need for exploring innovative therapeutic agents, which can help in the prevention of cancer. In addition, mortality rate as well as cost of treatment in breast cancer has a financial burden on patients. Therefore, searching for a natural and safe way of chemoprevention to reduce the cost of breast cancer management is needed. In recent years, nutraceuticals are considered as one of the approaches to counter several chronic diseases including cancer [6]. Nutraceuticals are described as any food or food derived products which help in maintenance of health as well as the prevention of disease. Nutraceutical properties of plants or plant derived products represent a clear link between ‘nutrients’ and ‘pharmaceuticals’ [7,8]. Therefore cancer prevention with nutraceutical potential biomolecules such as carotenoids (lutein) derived from okra could prove valuable. Okra (Abelmoschus esculentus L.) is one of the most widely utilized vegetables. Okra is been traditionally used not only as a vegetable but also been recognized in Indian traditional medicine usually in the form of infusions. Currently, okra is been used not only for its nutritional values but, also for its nutraceutical and therapeutic properties owing to presence of various bioactive compounds such as Polysaccharide, Rhamnogalacturonan, Lectins, Pectin, Polyphenolic compounds, Quercetin derivatives and epigallocatechin, flavonoids, carotenoids lutein and their associated bioactivities [2,9]. Previous studies reported the concentration of lutein in fresh okra pods to be 347µg-390µg/100g [10,11]. Carotenoids are primarily plant-derived lipophilic pigments with poly isoprenoid structures [12]. According to previous reports, it has been highlighted that women having high intake of dietary carotenoids, such as zeaxanthin, lycopene, carotene and lutein had considerably a low rate of breast cancer. Additionally in comparison to carotene, carotenoid lutein has been reported with no known toxicities, even in individuals who have consumed it at therapeutic dose [13]. Lutein is chemically (3R,3 R,6 R)-β-carotene-3-3 -diol), a oxygenated carotenoid isolated from plant or plant products such as okra [14]. Research articles suggest that lutein has a wide range of functions such as anti-inflammatory, growth inhibitory activity, cytotoxic activity in various cancer cell lines and animal models, preventing atherosclerosis, age-related macular degeneration [15], inhibits the activation of NF-κB [16], a enhancement of the immune system and as an antitumor activity [17]. In addition, it has been reported that lutein has antioxidant activity that inhibits the proliferation and angiogenesis of tumor cells [18].

Results: Out of 28 studies, 7 research articles fulfilled the inclusion criteria. Meta-analysis data indicated that, a lutein concentration at ≥1 µM was able to reduce the MCF-7 cell viability of 59.837 with a 95% confidence interval (CI): 48.331 to 71.343. Additionally, a forest plot of the cumulative studies also indicated that impact of lutein concentration to reduce the MCF-7 cell viability was around 60%. Moreover, the I² value of lutein was 74%, which is a considerable heterogeneity.

Conclusion: Therefore, based upon the meta-analysis data, the conclusion is that dietary lutein supplementation and fortification of food with clinical data could be an approach to develop a nutraceutical product for preventive, as well as for adjunct therapeutic purposes in various breast cancer subtypes.

Keywords: Anticancer; Breast Cancer Cell line; Carotenoids; Lutein; MCF-7; Nutraceuticals; Okra.
studies reported that, once lutein is isolated from the plant, it is biologically active in either the ester or the free form is consumed either in its free form or bound with proteins or esterified as monoesters and di-esters. Because studies reported that, once lutein is isolated from the plant, it is biologically active in either the ester or the free form. Because of its hydroxylated terminal rings, it does not meet nutritional importance as a pro-vitamin A molecule, unlike β-carotene [4,19]. Several case-control studies and combination analysis of cohort studies, indicated that intake of plant foods or its biomolecules such as lutein, reduces the risk of breast cancer [4]. Various studies have revealed lutein selectively inhibit the breast cancer cell line MCF-7. The MCF-7 cell line was developed from a pleural effusion at the Michigan Cancer Foundation in 1973, which is currently the most commonly used xenograft model of breast cancer [20]. Despite its origin from the metastases of an advanced tumor, the cell line is noninvasive and represents a model of early-stage disease due to the presence of functional ER and estrogen dependence for growth both in vitro and in vivo [20]. Therefore, this study was aimed to perform a meta-analysis of okra-derived biomolecule lutein against the MCF-7 breast cancer cell lines and to describe the potential for the development of lutein based nutraceutical.

2. MATERIALS AND METHODS

2.1 Identification Strategy and Eligibility of Applicable Studies

Google Scholar, EMBASE, Web of Science and PubMed were searched to explore a well-matched and peer reviewed research articles. Combinations of keywords such as “Lutein”, “Okra derivative lutein” “Anticancer activity”, “Breast cancer cell lines”, and “MCF-7” were searched for the duration of 2000 to 2020. Studies showing presence of lutein in okra, lutein for its anticancer activity, lutein more specifically for breast cancer cell lines as well as more specific to MCF-7 cell lines were evaluated. The published studies corresponding to the stated eligible standards were retrieved and encompassed in the present study [4, 5, 13, 21,22,23].

2.2 Inclusion and Exclusion Criteria

The following criteria were used for selection of studies for the meta-analysis, a) Any studies showing the presence of lutein in okra, b) Studies showing lutein as anticancer agent, c) Any studies showing lutein activity against breast cancer cell, d) Studies showing lutein against MCF-7 cancel cell lines. All the article retrieved were presented in English. Furthermore, the articles where duplication of data was found, review article and data which was not specific to lutein activity against MCF-7 cell lines were excluded from the study. A designed PRISMA flow chart is presented in Fig. 1 to depict exclusion and inclusion criteria.

2.3 Data Extraction and Quality Assessment

A standard protocol was used by three of the coauthors for data extraction and procedural quality assessment in duplicate. Data accurateness was validated using the data collection form according to inclusion/exclusion protocols as describe above. The incongruent items were fully debated to reach a conclusion. The characteristics summarized from the selected studies were the name of the first author and the year of publication.

2.4 Statistical Analysis

The statistical analyses were performed using Comprehensive Meta-Analysis (CMA) Version 2 software program (Biostat, USA) [24]. The p < 0.05 was fixed for considering the statistical significance. All the p-values were two sided. A random-effects method was used for the analysis of data. A 95% confidence interval was calculated and two-sided p-values for each outcome. For efficient testing of the heterogeneity, $I^2$ statistics was also employed [ 25, 26]. Egger’s linear regression test was used to estimate funnel plot asymmetry [ 27, 28].

2.5 Publication Bias Diagnosis and Heterogeneity Evaluation

The publication biasness present in the included studies was checked by funnel plot asymmetry. A p-value of < 0.05 was fixed for considering the significant publication bias. In addition, all the inhibition of cell viability against lutein were included in this meta-analysis were checked for heterogeneity using $I^2$ statistics.

3. RESULTS & DISCUSSION

3.1 Characteristics of the Article Retrieved

A total of 103 articles retrieved comprising of keywords such as lutein present in okra, lutein
anticancer activity, lutein against breast cancer cell lines and lutein against MCF-7 cell lines were recovered from the PubMed, EMBASE, and Google scholar databases. The recovered literature was screened by titles, abstracts, and the full texts analysis for its relevance. Identified articles were further analyzed and screened for their suitability for meta-analysis consideration. To find any additional relevant article, the reference lists of all the retrieved articles were screened for any and supplementary articles. Studies showing lutein anticancer activity other than MCF-7 cell lines were excluded. A strict principle was followed in article searches. Only those case–controls or cohort studies which included MCF-7 cell line activity against the lutein bioactive were considered. Following the thorough selection and strict inclusion and exclusion criteria, seven original articles were considered relevant and were added in the present analysis. Which is presented in the form of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

3.2 Publication Bias

The Begg’s funnel plot and Egger’s test were implemented to examine the publication bias among the included studies as presented in Fig. 2. It was found that 3 publications were out of the symmetry of the funnel, which indicates that publication biasness was present in this study. However, one publication was completely outside the funnel (outlier), and 2 publications were outside the symmetry but close to the funnel diagram.

3.3 Test of Heterogeneity

Heterogeneity between studies was assessed using the Higgins’ I² statistics. The value of I² was 74%, which is a considerable heterogeneity as presented in Fig. 3.

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**Fig. 1.** PRISMA Flow diagram showing the identification and studies selection chart (inclusion/exclusion) for the presented meta-analysis
Fig. 2. Funnel plot showing publication biasness, where x axis represent mean difference (MD) and y axis standard error (SE), each open circle denotes a study included in the meta-analysis

3.4 Meta-analysis of Lutein against Breast Cancer Cell Lines

Meta-analysis was performed using Comprehensive Meta-Analysis (CMA version 2) Software. A random-effects method was used for analysis of the data. A 95% confidence interval and two-sided p-values for each outcome were calculated. Meta-analysis results suggested that, lutein at 1 µM concentration would be effective in reducing MCF-7 cells viability with a standard mean difference of 59.837; at 95% confidence interval: 48.331 to 71.343. Heterogeneity between studies was assessed using the Higgins’ I² statistics. The value of I² was 74%, which is a considerable heterogeneity. The p value of the test was found to significant at p<0.001. Most of the studies are in left side of the plot (Fig. 3), which indicates that the study favors the experimental group. Horizontal line (Fig. 3) represents the number of study and the mid-point of the box represents the point effect estimate, and the mean effect estimate for each study. The area of the box represents the weight given to the study. The diamond presented in Figs. 3 and 4 represents the overall effect. Furthermore, the forest plot of the cumulative studies (Fig. 4) indicates that impact of lutein concentration in reducing the MCF-7 cells viability ie; 60%.

It has been revealed by several studies that dietary lutein intake is positively associated with a decrease in the number of breast cancer risk and results of this study were consistent with the previous one [4]. Moreover, findings of this study suggest that lutein at a specific concentration, reduces the cell viability of MCF-7 cell lines. Along with confirming this, the current study showed a high concentration of lutein in human diets would validate the development of lutein based nutraceuticals and functional food and could be an ideal supplement to prevent the breast cancer rate. Moreover, further clinical studies should be carried out regarding the newly developed nutraceutical formulations. With the best possible efforts made, it was found that the present meta-analysis study is the first report exploring the best possible lutein concentration for inhibiting the cell viability. The collective outcomes of this meta-analysis present that 1µM concentration of lutein affects the carcinogenesis in MCF-7 cell lines. This points out that lutein could be used as a potential bioactive compound for the development of nutraceutical products. While interpreting the results of the current meta-analysis, few limitations were identified, such as studies that were published in English. Moreover, only data cited by the designated databases, i.e. PubMed, Google scholar, and EMBASE were included). A possibility exists that pertinent studies may have been missed that have been published in languages other than English and cited elsewhere. In addition, this study showed an important aspect for the future development in food and pharmaceutical industries. First, since the current study is free from publication bias, it could be considered statistically robust. Second, the application of the stringent data extraction strategy based on manual searches as well as computer assistance, make it a dependable deduction.
Fig. 3. Forest plot showing effect of lutein concentration against MCF-7 cell lines inhibition

Fig. 4. Cumulative studies of lutein concentration impact against MCF-7 cell lines

4. CONCLUSION & FUTURE PROSPECTIVE

The meta-analysis reinforces the evidence that lutein has correlation with existed literature against the MCF-7 cell lines. Moreover, based upon the meta-analysis it is concluded that lutein had a significant effect in reducing the MCF-7 cells viability in a dose-dependent manner. Therefore, dietary lutein supplementation or fortification could be an approach to develop a nutraceutical and functional food product for the preventive, and for adjunct therapeutic purposes in human breast cancer. However, in vivo studies needed to validate the lutein concentration to explore the effectiveness, safety and efficacy in breast cancer patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021;71(3):209-249. Available:https://doi.org/10.3322/caac.21660.

2. Elkhalifa A, Alshammari E, Adnan M, Alcantara JC, Awadelkareem AM, Eltoum...
NE, Mehmoond K, Panda BP, Ashraf SA. Okra (Abelmoschus esculentus) as a Potential Dietary Medicine with Nutraceutical Importance for Sustainable Health Applications. Molecules. 2021a;26 (3):696. Available:https://doi.org/10.3390/molecules 26030696

3. Jazieh AR, Da’ar OB, Alkaiyat M, Zaatreh YA, Saad AA, Bustami R, et al. Cancer Incidence Trends from 1999 to 2015 And Contributions of Various Cancer Types to the Overall Burden: Projections to 2030 And Extrapolation of Economic Burden in Saudi Arabia. Cancer Management and Research. 2019;11:9665-9674. Available:https://doi.org/10.2147/CMAR.S22667

4. Kavalappa YP, Gopal SS, Ponesakki G. Lutein Inhibits Breast Cancer Cell Growth by Suppressing Antioxidant and Cell Survival Signals and Induces Apoptosis. Journal of Cellular Physiology. 2021;236:1798-1809. Available:https://doi.org/10.1002/jcp.29961

5. Li Y, Zhang Y, Liu X, Wang M, Wang P, Yang J, et al. Lutein Inhibits Proliferation, Invasion and Migration of Hypoxic Breast Cancer Cells Via Downregulation of HES1. International Journal of Oncology. 2018; 52(6):2119-2129. Available:https://doi.org/10.3892/ijo.2018.4332.

6. AIAli M, Alqubaisy M, Aljaafari MN, AIAli A O, Baqais L, Molouki A, A bushelaibti A, Lai, KS, Lim SE. Nutraceuticals: Transformation of Conventional Foods into Health Promoters/Disease Preventers and Safety Considerations. Molecules. 2021;26 (9):2540. Available:https://doi.org/10.3390/molecules 26092540

7. Ashraf SA, Adnan M, Patel M, Siddiqui AJ, Sachidanandan M, Snoussi M. Fish-based Bioactives as Potent Nutraceuticals: Exploring the Therapeutic Perspective of Sustainable Food from the Sea. Marine Drugs. 2020a;18(5):265. Available:https://doi.org/10.3390/md18050265

8. Ashraf SA, Elkhalifa A, Siddiqui AJ, Patel M, Awadelkareem AM, Snoussi M, et al. Cordycepin for Health and Wellbeing: A Potent Bioactive Metabolite of an Entomopathogenic Cordyceps Medicinal Fungus and Its Nutraceutical and Therapeutic Potential. Molecules. 2020b;25(12):2735. Available:https://doi.org/10.3390/molecules 25122735

9. Elkhalifa AEO, Al-Shammari E, Adnan M, Alcantara JC, Mehmoond K, Eltoum NE, et al. Development and Characterization of Novel Biopolymer Derived from Abelmoschus esculentus L. Extract and Its Antidiabetic Potential. Molecules. 2021b;26(12):3609. Available:https://doi.org/10.3390/molecules 26123609

10. Aruna G, Mamatha B, Baskaran V. Lutein Content of Selected Indian Vegetables and Vegetable Oils Determined by HPLC. Journal of Food Composition and Analysis. 2009;22:632-636. Available:https://doi.org/10.1016/j.jfca.2009.03.006

11. American Molecular degeneration Foundation; www.macular.org; 1998-2010. Lorenzo JM, Munekata PE. Dietary Carotenoids for Reduction of Cancer Risk. Studies in Natural Products Chemistry. 2016;223–251. DOI:10.1016/b978-0-444-63932-5.00006-1

12. Gong X, Smith JR, Swanson HM, Park L P. Carotenoid Lutein Selectively Inhibits Breast Cancer Cell Growth and Potentiates the Effect of Chemotherapeutic Agents Through ROS-Mediated Mechanisms. Molecules. 2018;23(4):905. Available:https://doi.org/10.3390/molecules 23040905

13. Calvo MM. Lutein: A Valuable Ingredient of Fruit and Vegetables. Critical Reviews in Food Science and Nutrition. 2005;45(7-8):671–696. DOI:10.1080/10408690590957034

14. Lima VC, Rosen RB, Farah M. Macular pigment in retinal health and disease. International Journal of Retina and Vitreous. 2016;2:19. Available: https://doi.org/10.1186/s40942-016-0044-9

15. Tan BL, Norhaizan ME, Liew WPP, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Frontiers in Pharmacology. 2018;9:1162. DOI: 10.3389/fphar.2018.01162

16. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. Carotenoids: biochemistry, pharmacology and treatment. British Journal of Pharmacology. 2017;174(11):1290–1324.
Available:https://doi.org/10.1111/bph.13625

18. Chang J, Zhang Y, Li Y, Lu K, Shen Y, Guo Y, Qi Q, Wang M, Zhang S. Nrf2/ARE and NF-κB Pathway Regulation May be the Mechanism for Lutein Inhibition of Human Breast Cancer Cell. Future Oncology. 2018;14(8):719-726. Available:https://doi.org/10.2217/fon-2017-0584

19. Ashraf SA, Al-Shammari E, Hussain T, Tajuddin S, Panda BP. In-vitro Antimicrobial Activity and Identification of Bioactive Components Using GC-MS of Commercially Available Essential Oils in Saudi Arabia. Journal of Food Science and Technology. 2017;54:3948-3958.

20. Welsh JE. Animal Models for Studying Prevention and Treatment of Breast Cancer, Editor(s): P. Michael Conn, Animal Models for the Study of Human Disease, Academic Press, 2013;997-1018, ISBN9780124158948, Available:https://doi.org/10.1016/B978-0-12-415894-8.00040-3.

21. Sowmya PRR, Arathi BP, Vijay K, Baskaran V, Lakshminarayana R. Astaxanthin from Shrimp Efficiently Modulates Oxidative Stress and Allied Cell Death Progression in MCF-7 Cells Treated Synergistically with β-carotene and Lutein from Greens. Food and Chemical Toxicology; 2017. DOI: 10.1016/j.fct.2017.05.024.

22. Vijay K, Sowmya PR, Arathi BP, Shilpa S, Shwetha HJ, Raju M, et al. Low-dose Doxorubicin with Carotenoids Selectively Alters Redox Status and Upregulates Oxidative Stress-mediated Apoptosis in Breast Cancer Cells. Food and Chemical Toxicology. 2018;118:675-690. DOI: 10.1016/j.fct.2018.06.027.

23. Soon-Park S, Chew BP, Wong TS. Dietary Lutein from Marigold Extract Inhibits Mammary Tumor Development in BALB/c Mice. The Journal of Nutrition. 1998;128(10):1650–1656. Available:https://doi.org/10.1093/jn/128.10.1650.

24. Borenstein M, Hedges L, Higgins J, Rothstein HR. Comprehensive meta-analysis (version 2) [computer software]. Englewood: Biostat; 2005.

25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557-60.

26. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials. 1986;7:177-88.

27. Egger M, Davey-Smith G, Schneider M, Minder C. Bias in Meta-analysis Detected by a Simple, Graphical Test. BMJ. 1997;315:629-34.

28. Mandal RK, Raish M, Jawed A, Wahid M, Dar SA, Lohani M, et all. Meta-analysis Reveals no Correlation of Caveolin-1 G14713A (G>A) Gene Polymorphism with Increased Cancer Risk in Taiwanese Population. International Journal of Health Sciences. 2018;12(3):3-9.