e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(6); 215-223

Original Research Article

Lutein Mediated Inhibition of Ovarian Cancer Cell Proliferation In-Vitro

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Received: 25-03-2024 / Revised: 23-04-2024 / Accepted: 26-05-2024

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Conflict of interest: Nil

Abstract:

Purpose: There is little evidence supporting lutein's, carotenoid, anticancer effects on ovarian cancer cells. The prognosis for ovarian cancer is still not great, particularly in places with limited resources. For this reason, it's critical to keep tracking the incidence of ovarian cancer in order to pinpoint disparities in the disease's management.

Methods: Various amounts of carotenoids were applied to cancer cells at 37°C in a CO2-containing 5% incubator. Cell proliferation was measured using the MTT test, and the anti-proliferative activity against cancer cells was evaluated using IC_{50} values.

Results: After 24 hours of incubation, lutein (IC_{50} = 0.77 µM) was more effective on PA-1 cells (lower IC_{50}) than it was after 48 hours (IC_{50} = 1.62 µM).

Conclusion: This study found that lutein can cause cancer cells to die and impede their ability to proliferate.

Keywords: Cancer, Carotenoid, Cell Proliferation Assay, IC_{50}, Lutein, Tamoxifen.

Introduction

The prognosis for ovarian cancer is still not great, particularly in places with limited resources. For this reason, it's critical to keep track of the incidence of ovarian cancer in order to pinpoint disparities in the disease's management. According to a GLOBOCON 2020 study, there were 207,000 fatalities and around 313,000 new cases of ovarian cancer reported globally [1]. The carotenoids lutein and zeaxanthin (ZXN) in human eyes function as antioxidants and UV protection. The blood contains large concentrations of these. These carotenoids' hydroxyl groups regulate their function [2-4].

A study discovered that a higher prevalence of persistent HPV infection was linked to a lower consumption of lutein, ZXN, β-cryptoxanthin, and vitamin C [5]. Carotenoids, tocopherols, and retinol may offer protection against cervical dysplasia and HPV persistence [6]. According to another research, that the consumption of lutein and ZXN decreased the incidence of larynx cancer by 17% and the risk of pharynx cancer by 18% in a trial that included nearly 18,000 people who had mouth, throat, and laryngeal cancer [7]. Li et al. (2018) [8] examined the effects of different concentrations of lutein on the migration, apoptosis, and viability of MDA-MB 157 and MCF-7 cells. Doses of 5, 10, 20, 40, 80, and 120 µM were used. Researchers found that lutein reduced HES1 gene expression via the Notch signaling system, which in turn reduced ROS generation, which in turn reduced the migration and proliferation of breast cancer cells. These results raise the prospect that lutein may find use as an anticancer treatment approach for breast cancer in the future. Le Marchand et al. (1993) [9] found that those with diets rich in lutein, β-carotene, and α-carotene were less likely to develop lung cancer.

Additionally, the study showed that eating a variety of veggies provided greater protection against illness than eating only meals high in one kind of carotenoid. Research using a meal frequency questionnaire was carried out in Northern Italy, involving 743 control subjects and 304

instances of squamous cell esophageal cancer. An odds ratio of 0.4 indicated a statistically significant adverse relationship between lutein+ZXN consumption and esophageal cancer risk. Franceschi et al., [10] A comparable survey carried out in Korea discovered that a greater consumption of total dietary carotenoids was linked to a lower incidence of stomach cancer in women. Kim et al. [11] found no evidence of a significant correlation between the risk of stomach cancer and dietary lutein/ZXN. HT-29 cells, which are malignant cells and normal colon cells (CCD 841 CoTr), were treated with 1, 5, and 10 µM of ZXN and lutein respectively. Both cell lines contained carotenoids, according to fluorescence microscopy, whereas carcinoma cells had a greater percentage of carotenoid molecules in monomeric form. According to the study's findings, lutein+ZXN decreased HT-29 cell survival without changing CCD 841 CoTr cell toxicity [12]. Among all participants in a food history questionnaire-based case-control research, lutein/ZXN (P=0.021) demonstrated an inverse correlation with renal cancer [13]. Similar research that was carried out in a number of different Italian locales discovered a strong inverse link (OR = 0.6; 95% CI: 0.5-0.8) between lutein/ZXN and the risk of ovarian cancer [14]. Considering bits of evidence of the anticancer effects of carotenoid, we examined the anticancer qualities of lutein on ovarian cancer cells (PA-1) compared to the conventional anticancer medicine tamoxifen (Tx).

Materials and Methods

PA-1 cells, Tx (purity 98%, analytical grade), and lutein (≥95%) were obtained from NCCS, Pune, India and Sigma Aldrich, respectively. The ethics committee gave its approval before any experiment was conducted. The PA-1 cells were grown in a specific environment: a 37°C incubator with a humidifier and 5% CO2 gas. The growth media used for the cells was DMEM with 10% FBS and 1% penicillin-streptomycin. After the pelleting procedure was finished, the cells were transferred onto 96-well plates using a pipette. For the purpose of inoculating the cells, 100 microliters of each concentration of the drug preparation that had been serially diluted (lutein, Tx) was utilized. For 24 and 48 hours, the cells were exposed to different drug concentrations (20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.078 µM), with each concentration being examined in triplicate.

MTT was employed in order to determine the cytotoxic impact that Tx and lutein had on PA-1. This decision was made in line with the directions provided by the manufacturer. After the incubation period had elapsed, 100µl of DMSO was added to each well in order to dissolve the formazan crystal that had formed as a consequence of the reaction. Additionally, the assay fluid that contained the MTT reagent was discarded. Absorbance was measured at 570 nm using a BioRed Lab Model 3550 microplate reader. The reference wavelength was 650 nm, and the reader was used to measure absorbance.

Determination of half maximal inhibitory concentration (IC50): A sigmoid curve was fitted to the dataset of the PA-1 cell line and drug treatment pair individually at 24 and 48 hours. The data point was fitted using the modified Hill equation.

Statistical Analysis: Using the MTT test, the study looked at how lutein and Tx affected the viability of PA-1 cells. The impact of time intervals, treatment intensities, and treatment kinds on cell viability was assessed statistically. Various time periods and medication types were compared at each treatment level using Bonferroni-adjusted analysis. Post hoc Tukey HSD analyses were also performed. There was a $p<0.05$ significance threshold used. The analysis of the data was done with SPSS 21. In order to assess the anti-proliferative potency, IC50 values were produced. Graph Pad Prism 8 was used to graph the findings.

Results

MTT cell proliferation assay

Effects of Lutein on PA - 1 cells at 24 and 48 hrs (Fig: 1)

- The effects of time periods and treatment levels on the percentage viability of cells were found to interact statistically significantly when lutein therapy was applied ($F_{(9, 40)} = 3.637$, $p = 0.002$, partial $\eta2 = 0.450$).
- Compared to 48 hours, the mean percentage viability of cells treated with lutein at the following doses was lower: 5 µM ($p=0.012$), 1.25 µM ($p<0.001$), 0.625 µM ($p=0.018$), 0.3125 µM ($p=0.016$), and 0.15625 µM ($p<0.001$), respectively (Fig. 18). Interestingly, the drop at 10 µM was more noticeable after 48 hours of incubation than it was after 24 ($p=0.032$).
- At the 24- and 48-hour time periods, there was a highly significant effect of different medication concentrations on the percentage vitality of cells. ($F_{(9, 20)} = 5.81$, $p< 0.001$ for 24 hours, and $F(9, 20) = 5.59$, $p< 0.001$ for 48 hours).
- All drug doses substantially decreased the percentage viability of PA-1 cells compared to controls (20 µM to 0.625 µM: $p< 0.01$; 0.3125 µM to 0.078 µM: $p< 0.05$), according to post hoc comparisons conducted after 24 hours. Conversely, post hoc analysis at 48

hours revealed a statistically significant decrease in viability compared to control, primarily with a 10 µM concentration (p= 0.002).

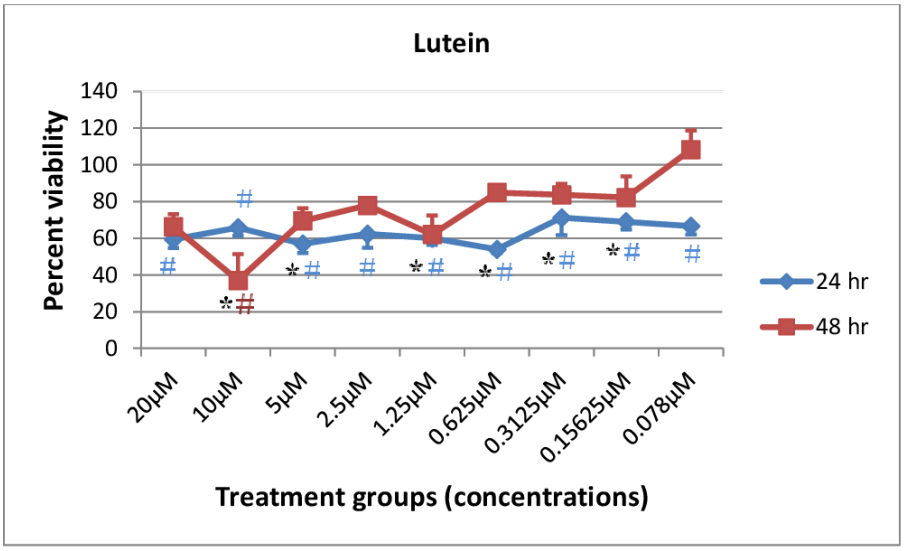

Figure 1: *significant difference b/w 24 and 48 hrs
significant difference b/w 24 hrs and control
significant difference b/w 48 hrs and control

Effects of Tx on PA - 1 cells at 24 and 48 hrs (Fig. 2)

- The variables of time points and treatment concentrations had an overall statistically significant interaction on the influence of the percentage vitality of malignant cells ($F_{(9, 40)} = 3.157$, $p = 0.006$, partial $\eta 2 = 0.415$).
- To be more precise, Tx at 10 µM ($p < 0.001$), 2.5 µM (= 0.023), 1.25 µM ($p = 0.008$), and 0.3125 µM ($p = 0.023$) dramatically inhibited the proliferation of PA-1 cells after 24 hours. Following a 24-hour incubation period, we observed a noteworthy impact of varying concentrations on the percentage viability ($F_{(9, 20)} = 5.74$, $p < 0.001$).
- The post-hoc test revealed that at 24 hours, 10 µM had a significantly lower viability percentage than control ($p = 0.017$).

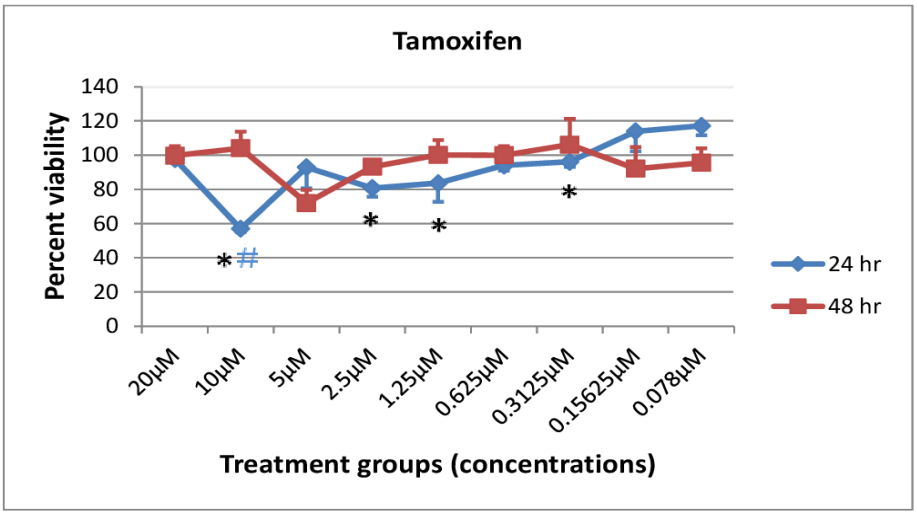

Figure 2: * significant difference b/w 24 and 48 hrs
significant difference b/w 24 hrs and control
significant difference b/w 48 hrs and control

Nevertheless, our experiment suggests that 48 hours of incubation was ineffective in regulating the proliferation of PA-1 cells ($F(9, 20) = 1.2$, $p = 0.35$).

Comparative effects of Lutein and Tx at 24 hrs (Fig. 3)

- After the cells were incubated for 24 hours, it was shown that the variables of drug kinds and treatment doses significantly interacted with the impact of %viability ($F_{(9, 40)}$ = 4.662, $p < 0.001$, partial $\eta^2 = 0.512$).
- The mean percentage viability of PA-1 cells was consistently lower and statistically significant (all $p<0.04$) than Tx for nearly all lutein concentrations (20 µM, 5 µM to 0.15625 µM). Only at 10 µM ($p>0.05$) did the difference between the two medications do not reach significance. This is a plausible indication that, at the 24-hour time point in our experiment, lutein had a greater overall anti-PA-1 cell action (anticancer effect) than Tx.

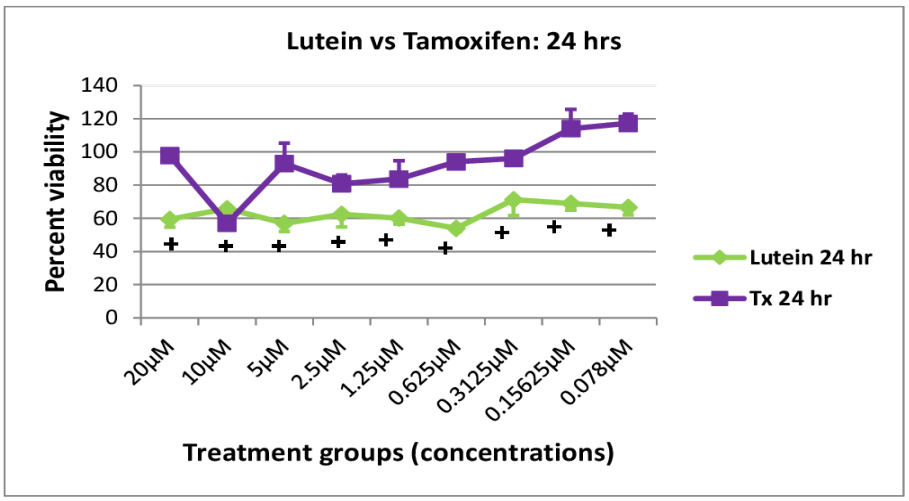

Figure 3: [+]Comparison between lutein and Tx at 24 hrs, P< 0.05

Comparative effects of Lutein and Tx at 48 hrs (Fig: 4)

- Lastly, a significant interaction between the drug types and treatment levels and the impact on %viability was also seen after 48 hours of incubation of the cells with the two medicines ($F_{(9, 40)}$ = 3.455, p = 0.003, partial $\eta^2 = 0.437$).
- Overall, the mean percentage vitality of PA-1 cells treated with lutein was higher than that of Tx; however, at some doses, namely 20 µM (p = 0.009), 10 µM (p < 0.001), and 1.25 µM (p = 0.003), lutein significantly reduced the percentage viability of PA-1 cells compared to Tx.

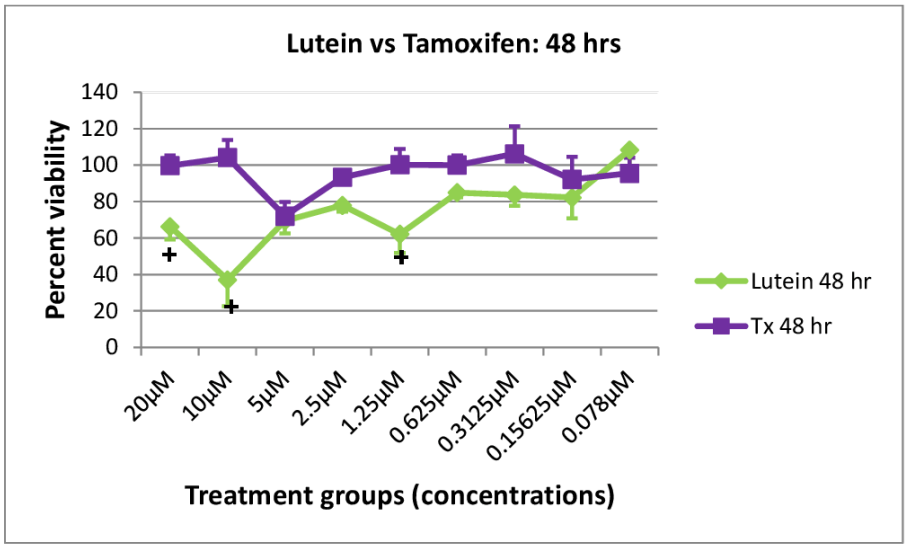

Figure 4: [+]Comparison between lutein and Tx at 48 hrs, P< 0.05

IC_{50}

By calculating IC_{50} values, the anti-proliferative efficacy of lutein on PA-1 cells was assessed. After 24 hours of incubation, lutein (IC_{50} = 0.77 µM, Fig. 5A) and Tx (IC_{50} = 2.63 µM, Fig. 6A) were shown to be more powerful on PA-1 cells (lower IC_{50}) in comparison to their respective 48-hour values (IC_{50} = 1.62 µM, Fig. 5B; IC_{50} = 10.77 µM, Fig. 6B).

This implies that the viability of PA-1 cells was lowered by lower drug doses during a 24-hour incubation period as opposed to a 48-hour one.

In this experiment, lutein was shown to be most effective against PA-1 cells at 24 hours (Fig. 7).

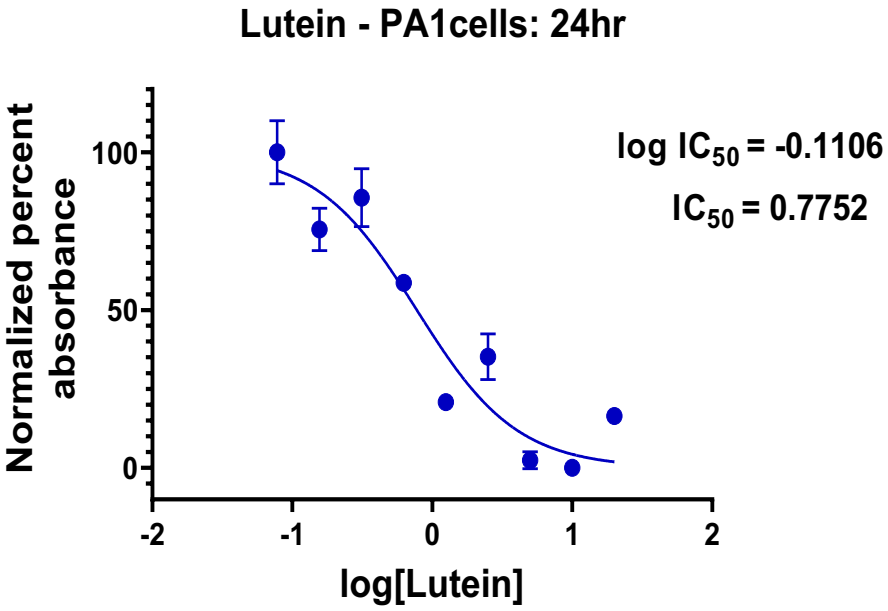

Figure 5A: The potency of lutein at 24 hrs

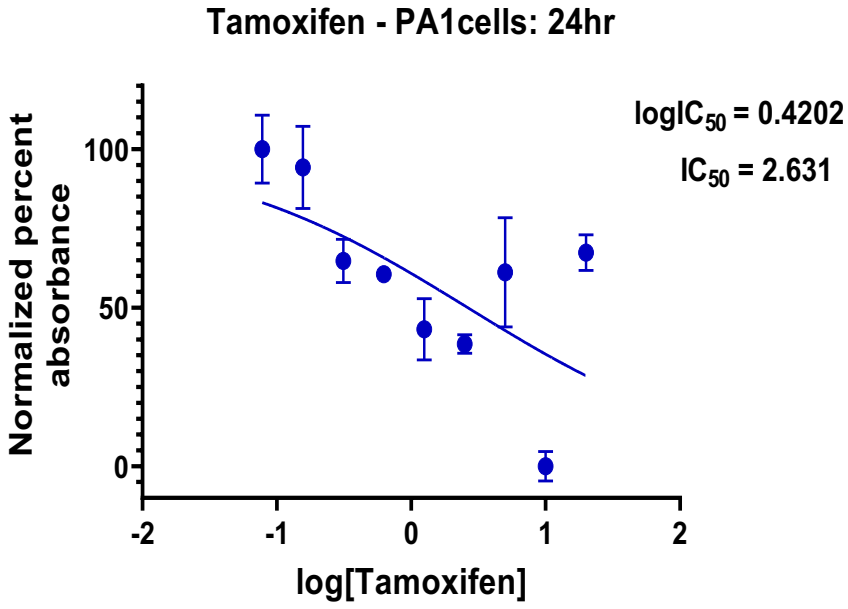

Figure 6A: The potency of Tx at 24 hrs

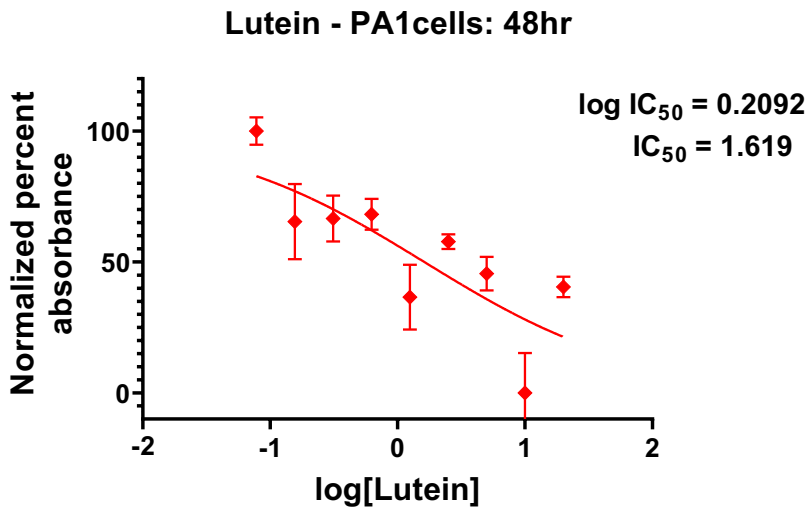

Figure 5B: The potency of lutein at 48 hrs

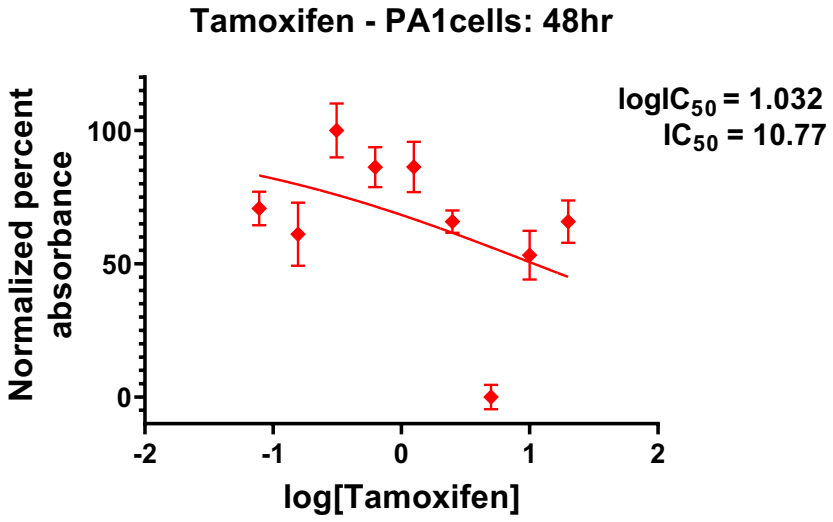

Figure 6B: The potency of Tx at 48 hrs

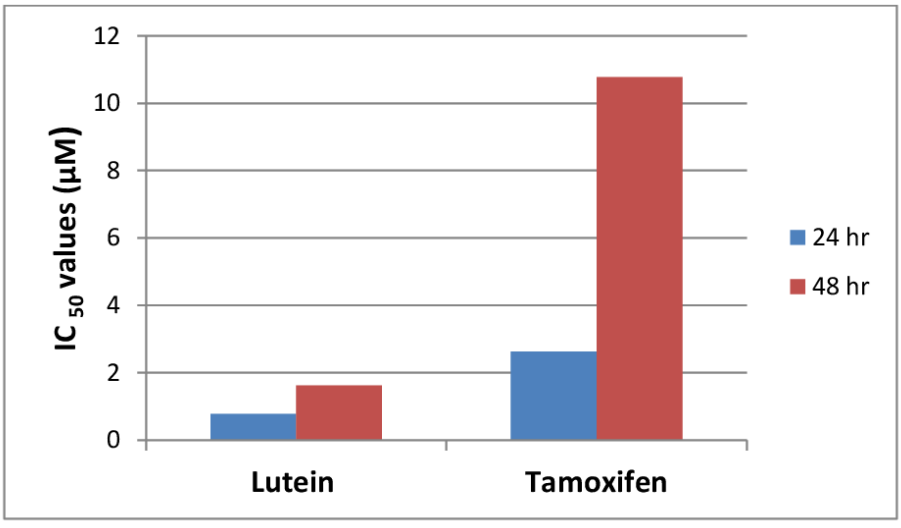

Figure 7: Comparison of IC_{50} across treatment groups

Discussion

Conversely, lutein is only found in naturally occurring foods like fruits and vegetables. There is lutein, which is a kind of carotenoid. Most people agree that it is safe to eat at different levels. To be considered normal, the quantities of lutein in the body range from 0.1 to 2.3 μM in the lungs, 100 to 1,000 μM in the macula, 0.1 to 1.23 μM in the plasma, and 0.1-3.0 μM in the liver. Research investigations have demonstrated that a higher probability of developing chronic illnesses like cancer is linked to a plasma lutein content below 1 μM [15,16].

Treatment of non-small cell lung cancer (NSCLC) cells with lutein halted cell cycle progression at the G0/G1 phase, leading to cell death. In NSCLC, lutein's anticancer effects were shown to work via producing DNA damage and activating the ATR/Chk1/p53 signaling pathway, which is associated with the inhibition of tumor development [17]. When 2023 rolled around, Zhang and colleagues By utilizing four distinct gastric cancer cell lines (AGS, MKN-74, MKN-1, and SNU-669) and one anticancer medication (etoposide), scientists examined how lutein impacted cancer cell apoptosis. Furthermore, they found out if lutein improved the ability of chemotherapy drugs to successfully kill cancer cells. It was concluded from the experiment results that 20μM of lutein caused cells linked to stomach cancer to die off and an increase in reactive oxygen species (ROS). The normal gastric mucosal RGM cells and gastric cancer cells, especially AGS, MKN-74, MKN-1, and SNU-668, were treated with a mixture of etoposide with a small dosage of lutein, specifically 5 μM. Evidence suggests that AGS cells and MKN-1 cells were considerably more susceptible to etoposide-induced cell death when administered lutein at a dose of 5 μM. In AGS cells, lutein activates NF-kB and apoptotic markers; it then promotes apoptosis by elevating ROS levels and activating NADPH oxidase. Lutein can increase the cell death caused by etoposide in AGS cells [18].

Compared to Tx, lutein had a stronger anticancer effect at the 24-hour point in the ongoing trials (Fig. 1). There was a significant difference in the vitality of PA-1 cells treated with lutein compared to controls after 48 hours. In addition, it should be mentioned that at certain lutein dosages—20 μM ($p = 0.009$), 10 μM ($p < 0.001$), and 1.25 μM ($p = 0.003$)—the vitality of PA-1 cells treated with lutein was significantly lower than that of Tx, as shown in Figure 4. In order to determine how effective carotenoids were, the IC50 values were measured at both 24 and 48 hours. Results showed that 24-hour incubation reduced PA-1 cell viability compared to 48-hour incubation when using lower doses of lutein (Figs. 5A, 5B) and Tx (Figs. 6A, 6B). After 24 hours, lutein showed its maximum effectiveness against PA-1 cells, taking everything into account (Fig.7).

HeLa cells were used to investigate the lutein's apoptotic activity using various administration methods. At 72 hours after incubation, lutein (25–100μM) administered via THF, DMSO, and FBS had an impact on cell viability of 54.1%, 58.6%, and 73.4%, respectively. Furthermore, according to Sowmya et al. (2015) [19], lutein did not cause cell necrosis at concentrations lower than 25 μM. Conversely, in the current investigation, lutein demonstrated its anticancer properties against PA-1 cells even at low doses (less than 20 μM).

Conclusion

The current study demonstrates that, when compared to the effects of Tx, lutein is more effective at 24 hours in suppressing cell growth and triggering apoptosis in PA-1 cells at nearly all doses. There have been several conceivable theories put out on the anticancer effects of carotenoids on ovarian and cervical cancer cells. Among these pathways is the P13/AKT pathway, which is essential for cervical cancer cell proliferation, cell cycle control, and apoptosis [20]. NF-kappa B activation is another way; this protein is known to have a role in a number of cancer processes, including inflammation, angiogenesis, and cell proliferation. Additionally, NF-kappa B stimulates the development of tumor cells and prevents apoptosis [21] (Ye et al., 2018). Furthermore, it has been discovered that lutein activates the intrinsic route in HeLa cells to cause ROS production and death [22].

The anticancer effects of lutein with its mechanistic understanding have been the subject of very few investigations employing cellular models. In particular, this work fills a vacuum in the literature by examining the impact of lutein on ovarian cancer cells. However, the particular methods and processes by which lutein controls the cell cycle suppresses cell proliferation, and induces yet to be identified.

The anticancer potential of lutein is associated with its robust antioxidant qualities, capacity to regulate inflammatory processes, and possible involvement in DNA repair pathways. Furthermore, lutein is more appealing for cancer prevention and therapy due to its exceptional capacity to build up in all bodily tissues and organs, including those impacted by cancer. Research has indicated that lutein may provide defense against a number of cancers, including skin, lung, breast, and colon cancer.

Additionally, different people may respond differently to lutein, and the best dosages and regimens are yet unknown. Consequently, more investigation is required to fully realize lutein's anti-cancer potential.

In conclusion, research on lutein's anticancer properties is fascinating and has intriguing ramifications for both cancer prevention and therapy. It is critical to remain up to date on the most recent advancements and discoveries in this fascinating topic as our comprehension of the processes behind lutein and its interactions with cancer continues to expand.

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