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FLAXSEED OIL AS A NOVEL ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR AGENT: ENHANCING CISPLATIN EFFICACY IN SQUAMOUS CELL CARCINOMA TREATMENT

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ABSTRAC

Squamous cell carcinoma represents a widespread form of cancer that demands new innovative and effective therapeutic strategies to address its growth concern. This research investigates whether flaxseed oil functions as a supplemental therapeutic agent which improves the effectiveness of cisplatin against squamous cell carcinoma (SCC) cell lines particularly using the OECM-1 cell line. The experimental design involved quantifying SCC25 cell growth rates by investigating epidermal growth factor receptor activity in 96-well plates as the primary means to study biological process activity. The cell treatment consisted of studying flaxseed oil and cisplatin alone and combined to determine their cytotoxic effects. The IC50 values represent the concentration of the compound required to inhibit 50% of the epidermal growth factor receptors (EGFR) activity in SCC25 cells. The statistical tests involving one-way ANOVA and paired sample t-tests allowed measurement of cell viability at 24 and 48 hours after treatment. Results indicate that cells treated with flaxseed oil experienced a major decrease in cell viability from 46.28 to 29.14 ($p = 0.007$) and received similar reductions as cells treated with cisplatin which declined from 23.10 to 16.22 ($p = 0.032$). A remarkable change from 35.06 to 20.76 ($p = 0.008$) was observed through combined administration. Mean values within the control group dramatically increased to reflect the success of the treatments applied. This research establishes that therapeutic potential of flaxseed oil when paired with cisplatin treatment for squamous cell carcinoma. The research indicates that implementing natural compounds alongside conventional therapies leads to superior therapeutic outcomes and diminished side effects. Research initiatives should concentrate on detailed investigations of synergistic processes alongside clinical performance assessments for combined cancer treatments in medical settings.

Keywords: *Flaxseed oil, Squamous cell carcinoma, Cisplatin, Epidermal Growth Factor Receptors, Natural Therapeutics.*

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INTRODUCTION

Bioactive compounds for medical use occur predominantly in natural products because of their extensive chemical diversity in nature. World Health Organization (WHO) defines medicinal plants through their capability to deliver therapeutic substances from any plant organ or serve as raw materials for chemo-pharmaceutical semi-synthesis. [(1)] During the past several decades scientists have devoted their efforts to extracting new natural substances from plants and microorganisms along with other organisms to evaluate their anticancer functions and their mechanisms of action. Scientists discovered a variety of anti-cancer medication through their research. [(2-4)]

The extraction product known as flaxseed oil originates from the seeds of Linum usitatissimum flax plant. Flaxseed oil provides health advantages because it contains omega-3 elements in addition to lignans, α-linolenic acid, proteins and fibers. The distinct nutrient profile of flaxseed oil outshines other plant-based oils since it demonstrates beneficial effects on cardiovascular health as well as anti-inflammatory properties and improved metabolic profiles and lower cancer risks.[(5-10)]

The epidermal growth factor receptor exists as a transmembrane glycoprotein which represents one member from the erbB family of tyrosine kinase receptors. Upon the attachment of EGFR to suitable ligands the autophosphorylation process initiates followed by receptor tyrosine kinase activation which triggers multiple signaling pathways involved in cellular proliferation regulation and differentiation together with survival maintenance. [(11-12)] Mutations (changes) in the EGFR gene produce higher than normal quantities of epidermal growth factor receptor proteins on specific cancer cell types. The abnormal processes result in faster cell division among cancer cells.[(13)]

The epidermal growth factor receptor (EGFR) shows elevated expression across non-small-cell lung cancer together with metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer and breast cancer. [(14-16)] EGFR activity shows different pathways to increase its expression through typical mutations and shortened segments inside its external domain. The outgrowth of multiple cancer-causing processes including continual cell division happens because EGFR mutations and truncations lead to independent receptor signaling. [(17)]

Among the many cancers cisplatin (cis diamminedichloroplatinum (II)) treats as a platinum-based chemotherapeutic agent it shows particular effectiveness against sequamous cell carcinoma. This agent attacks cellular compounds that include membrane phospholipids and thiol-containing peptides as well as diverse other cellular elements. The compound shows binding capability for both protein molecules as well as RNA that has not yet undergone transcription. Cisplatin requires DNA as its main essential target. The cellular entry of cisplatin enables it to replace one or two chlorine molecules with H2O molecules which creates permanent DNA damage.[(18-20)]

Dispite the role of cisplatin as an anti-cancer drug, the clinical use of cisplatin is often associated with severe side effects and the development of drug resistance. Therefore, there is a need to explore alternative or complementary therapeutic approaches to enhance the efficacy of cisplatin and reduce its toxicity. In this experiment, we investigated the effects of treatment of cultured sguamous cell carcinoma cell lines (SCC25) with flaxseed oil, cisplatin, and a mix of both (flaxseed oil and cisplatin) in order to investigate their mechanisms underlying changes in cell growth.

Materials and Methods:

Materials:

In this study we used the SCC25 cell line derived from human squamous cell carcinoma cells (American Type Culture Collection). Pure flaxseed oil stored in a dark glass container to block oxidation and protect the oil from damage and Cisplatin: PLATINOL-AQ® were used in this experiment along with the EGFR (ERBB) Human ELISA Kit v4a-ab100505.

Methodology:

Cytotoxicity Assay: [(21)]

The SCC25 cells received medium with DMEM supplemented by 10% FBS and penicillin-streptomycin at 1% and L-glutamine at 1% for culturing. The cells were cultivated in this environment which consisted of 37°C incubator with 5% CO2 levels and high humidity. The medium was changed every 2-3 days while cells were split upon reaching 80% confluence through the use of trypsin-EDTA solution. The experimental groups included:

1. The experimental group received no treatment exposure with both zero flaxseed oil and zero micrograms of cisplatin.
2. Flaxseed oil alone
3. Cisplatin alone
4. Combination of flaxseed oil and cisplatin at fixed ratios

96-well plates containing 1 × 10^4 SCC25 cells each to achieve full well adhesion before starting the experiment. The culture medium received a medium change to fresh DMEM which contained flaxseed oil and/or cisplatin at twenty-four hours. The cells spent an additional 24 hours along with 48 hours under laboratory conditions.

Measurement of Cell Viability

Cell viability measurements operated through the methyl tetrazolium (MTT) blue assay which detects the epidermal growth factor receptor EGFR activity of alive cells. Each day, 5 µl/well of a 5 mg/ml MTT solution was added to a replicate plate and after a 3h incubation the media was removed, the cells solubilized in 100 µl DMSO, and the absorbance measured at 450 nm. Each experiment was performed using six wells/condition and the average determined. The percent inhibition of growth compared to cells treated only with media was determined and the mean for three independent experiments was reported.

Calculation of IC50 Values

Calculate IC50 values using ROBONIK P2000 Elisa reader.

The reported IC50 is the average of all IC50 values collected on at least three different days, and the error value is the standard deviation.

GraphPad Prism software enabled data plotting for generating dose-response curves that subsequently led to IC50 values determination through nonlinear regression analysis.

Statistical analysis

All data were collected, calculated, tabulated and statistically analyzed using the following statistical tests. A normality test (Kolmogorov-Smirnov) was done to check normal distribution of the samples. Descriptive statistics was calculated in the form of Mean ± Standard deviation (SD). One-way ANOVAs was used to compare between groups in each variables under study. Bonferroni's as post hoc test was performed for the evaluation of statistical significances among the groups. Paired sample T test was used to compare between time intervals. P value ≤ 0.05 is considered be statistically significant. All Statistical analysis was performed using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp).

Results:

The table and figure (Table 1, and Fig.1) present mean values and standard deviations (SD) for the epidermal growth factor receptor activity in different treatment groups at 24 and 48 hours.

Intra-Group Analysis:

- **Flax Oil/SCC25** shows a significant decrease in mean scores from 46.28 to 29.14 at 24 and 48 hours, respectively ($p = 0.007$).
- **Cisplatin/SCC25** indicates a reduction from 23.10 to 16.22, with a significant p-value (0.032).
- **Mix/SCC25** also demonstrates a notable decline from 35.06 to 20.76 ($p = 0.008$).
- **Control/SCC25** has a higher mean value that increased from 106.70 to 125.03, which is statistically significant ($p = 0.001$).

Inter-Group Analysis:

- There are clear differences among groups at both time points, as indicated by the ANOVA results ($p < 0.001$ for both). The control group consistently outperforms the other groups in mean values at both time points, suggesting a significant effect of the treatments.

Overall, both intra- and inter-group comparisons highlight significant variations, indicating the relevance of the treatments evaluated.

Table 1: comparison between groups at different times						
Groups	24hr		48hr		Paired T test	P value
	Mean	**SD**	**Mean**	**SD**		
Flax Oil/SCC25	**46.28**[b]	1.39	**29.14**[b]	1.62	***11.91***	***0.007****
Cisplatin/SCC25	**23.10**[d]	1.54	**16.22**[d]	2.08	***5.43***	***0.032****
Mix/SCC25	**35.06**[c]	2.03	**20.76**[c]	2.11	***11.34***	***0.008****
Cont.SCC25	**106.70**[a]	2.96	**125.03**[a]	3.26	***28.48***	***0.001****
ANOVA test	***963.891***		***1458.38***			
P value	***<0.001****		***<0.001****			

Table 1: Results of Compounds Effects on EGFR in SCC25 Cells

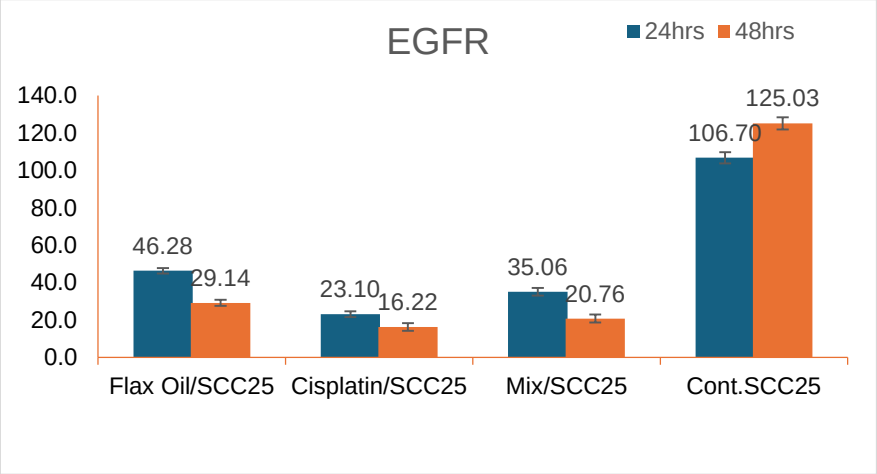

Figure 1: Bar graph of EGFR activity in SCC25 Cells of control, Flax oil, Cisplatin, and Mix (Flax oil & Cisplatin) after 24 and 48 hours.

Discussion:

The study results demonstrate significant information about how flaxseed oil with cisplatin and their joint application affect cell growth of cultured squamous cell carcinoma (SCC25). The decline of cell viability results observed in the treatment groups indicates promising potential for these agents in cancer therapy especially when used to lessen the negative effects of cisplatin chemotherapy. This section examines our findings' clinical importance together with their possible technical mechanisms and their significance for developing better cancer treatment strategies across the board.

Since epidermal growth factor receptors (EGFR) are essential for controlling cell growth, proliferation, and survival; [22-23] their activity in SCC25 cells was measured in order to analyze the cell growth rates. A number of malignancies, including squamous cell carcinoma (SCC), have been linked to the onset and spread of aberrant EGFR signaling activity. EGFR targeting has therefore become a viable therapeutic approach for the management of SCC. [24-25]

Flaxseed oil exposure decreased mean cell viability from 46.28 to 29.14 during 24 to 48 hours with statistical significance ($p = 0.007$). Incubation with flaxseed oil caused a major decrease in cell survival of SCC25 cells. The bioactive components in flaxseed oil prevent cancer by the induction of apoptosis and inhibition of cell proliferation. [26-29] Furthermore, the observed effects of flaxseed oil on cellular proliferation suggest a potential inhibitory role on epidermal growth factor receptor (EGFR) signaling, indicating its promise as a prospective anti-EGFR therapeutic agent.

The viability of SCC25 cells diminished from 23.10 to 16.22 ($p = 0.032$) under Cisplatin treatment conditions. Cisplatin stands as a commonly used cancer treatment drug because it creates DNA cross-links that prevent DNA replication and activate programmed cell death. The treatment of cancer with cisplatin faces considerable obstacles because patients can develop resistance to this therapy. [30] The present research indicates that cisplatin works effectively to reduce cell viability yet achieves weaker results than those found with flaxseed oil application. The observed reduced effectiveness of cisplatin treatment creates important questions about resistant processes while emphasizing the necessity of future research on combination therapeutic approaches to improve its efficacy.

Flaxseed oil in combination with cisplatin (Mix/SCC25) produced important results showing cell viability decreased from 35.06 to 20.76 ($p = 0.008$). The combined therapeutic drug action in the combination group became more effective likely because both agents operate through complementary mechanisms. The DNA-targeting properties of cisplatin seem to boost apoptosis but the chemotherapy inhibiting effect of flaxseed oil works through improved inflammatory pathway and oxidative stress management in cancer cells.[31-34] The co-treatment of cisplatin chemotherapy with flaxseed oil shows potential to defeat treatment resistance and result in better clinical results.

The control group cells showed substantially elevated mean cell survivability as the results jumped from 106.70 to 125.03 ($p = 0.001$). The study results demonstrate why treatment interventions are essential for SCC25 cell management since untreated cells maintained their growth pattern. Therapeutic agents demonstrated their crucial function in cancer cell growth inhibition through the analysis which presented clear differences between the control and treatment groups thus indicating the potential benefits of flaxseed oil and cisplatin in multidimensional cancer treatments.

ANOVA results demonstrate that the treatment groups displayed substantial variation statistically based on their differences which generated p-values less than 0.001 at both time points. The control group maintained constant viability throughout the tests which validated the testing procedures. The results strengthen future investigation into flaxseed oil combined with cisplatin because both show promise as therapeutic agents.

The experimental findings reveal important points about how flaxseed oil and cisplatin affect SCC25 cells. The effects of flaxseed oil on host signaling pathways that control cell growth and programmed death deserve special attention. Alpha-lipoic acid (ALA) shows the ability to affect genes that control cell cycle progression and cell death thus improving cisplatin chemotherapy responsiveness in cancer cells according to research findings. The anti-inflammatory components of flaxseed oil assist in generating a tumor environment unfavorable to cancer cell development and survival.[34-35]

Conclusion and Recommendations:
The present research demonstrates significant evidence which supports both flaxseed oil's anti-cancer potential and its ability to boost cisplatin's effectiveness against squamous cell carcinoma. Research demands additional investigation into mechanism of action studies as well as clinical evaluations because significant cell viability drops were noted with both agents along with their combined synergistic impact. The research for better cancer treatments continues to show promise in integrating natural products represented by flaxseed oil which offers positive potential for improving patient success while tackling drug-resistant challenges. Research needs to explore the fundamental mechanisms and test optimal therapy plans while conducting human trials to validate the research findings from this study. Our research about these compounds will help us create better methods for complete cancer treatment.

Conflicts of interest
The authors hereby state that this research work and manuscript production complied with ethical standards and none of the authors have any potential conflict of interest. We further declare that thisr esearch was not funded by any agency.

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