Original Article

Comparison of Sesamol and its Liposomal Nanocarrier in the Quest for Anti-Metastatic Agents in Colorectal Cancer Cell Line HT-29

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ABSTRACT

Objective: To evaluate the effects of sesamol alone and its liposomal nanocarriers in inhibiting the migratory potential of colon cancer cell line (HT- 29).

Study Design: Experimental Study

Place and Duration of Study: This study was conducted at the Department of Molecular Medicine, Ziauddin University, Clifton Campus, Karachi from October, 2022 to July, 2023.

Materials and Methods: The anti-migratory effects of sesamol, sesamol loaded liposomal nanocarriers and 5-Flourouracil on CRC cell line was evaluated through scratch assay, cells were seeded in 6 well plate and a wound was introduced using 10ul pipette tip on the layer of cells. The cells were then treated with compounds and observed under inverted microscope at different time intervals (0, 24, 48, 72 and 96 hours). Images were captured and the wound closure area was analyzed using image J software.

Results: The results of our study revealed that sesamol and its liposomal nanocarriers showed highly significant reduction in wound healing with p-value (<0.0001) up till 96 hours. While 5-FU has shown very highly significant results till 48 hours with p-value of (<0.0001) and after which it showed complete cytotoxicity.

Conclusion: Current study depicted significant anti-migratory effects of sesamol and its liposomal nanocarriers in CRC cell line HT-29, suggesting their anticipated role in impeding the metastasis of colorectal cancer.

Key Words: Sesamol, scratch assay, colorectal cancer cell line HT-29, cell migration.

Citation of article: Liaquat A, Ismail K, Usman S, Gul J, Shah R, Ismail MO. Comparison of Sesamol and its Liposomal Nanocarrier in the Quest for Anti-Metastatic Agents in Colorectal Cancer Cell Line HT-29. Med Forum 2023;34(9):46-50. doi:10.60110/medforum.340911.

INTRODUCTION

Colorectal Cancer (CRC) is the 3rd most common type of cancer worldwide and is the 2nd common cause of cancer related death(1). It is one of the leading cancer causing high mortalities globally(2). CRC is bit notorious for its metastatic potential and around 70% of patients with colorectal cancer are prone to develop hepatic metastasis during the course of their disease(3).

To deal with the limitations of conventional drugs in recent years researchers are very interested to search different polyphenols as these naturally occurring compounds display plentiful protective and therapeutic properties for health-related glitches. Comparatively lower toxicities and abundance of multiple beneficial features within the same compound permit the polyphenols to gain the researcher's attention for their anticipated medicinal values(4). Sesamol, a safe polyphenol derived from sesame seeds, has shown anti-proliferative, anti-metastatic and pro-apoptotic effects against various cancers(5). In HepG2 cell line sesamol has shown to decrease survival of cancer cells, stopped cell cycle, increased apoptosis and promoted loss of mitochondrial membrane potential(6). One study done on CRC cell line DLD-1, it causes inhibition of COX-2, reduces the prostaglandin E2 receptor expression levels thereby inhibit inflammation(7) Moreover in lung adenocarcinoma (SK-LU-1) cell lines, sesamol has shown promising anti-proliferative and pro-apoptotic activities(8)

Another development in the management of cancer is the use of nanotechnology, ultimate goal of which is to increase therapeutic efficacy and to reduce the adverse effects of the conventional drugs(9). At present, researchers are working on liposomal conjugation of natural compounds to increase their bioavailability,

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Received: August, 2023
Accepted: August, 2023
Printed: September, 2023

eventually to avail maximum therapeutic effects. For example liposomal ellagic acid a polyphenolic compound found in vegetables and fruits more effectively eliminated Cryptococcus neoformans infection compared to standard drug[10].

Keeping in view the above facts this study was design to evaluate and compare the anti-migratory potential of sesamol, sesamol loaded liposomal nanocarriers and standard chemotherapeutic agent 5-flourouracil on CRC cell line HT-29.

MATERIALS AND METHODS

Chemicals: Sesamol was purchased from Ambeed, Inc. company USA with a purity of 99.90%. MEM (Dulbecco's modified Eagle's medium), Pencillin-streptomycin, Fetal Bovine Serum, MTT (3-(4, 5-Diamethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) were purchased from Thermo Fisher Scientific (Life Tech). esamol loaded liposomal nanocarriers was provided by Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry at Karachi University. HT-29 colorectal cancer cell line was a gift from Dr.Azhar Hussain (Director, Laboratories, Biological and Biomedical Sciences at Aga Khan University, Karachi, Pakistan).

Cell Line Revival and Sub-Culturing of HT-29: or cell revival, cells were taken out from liquid nitrogen tank and immediately thawed in luke warm water for one minute, transferred to the falcon tube containing 9ml fresh media complete Dulbecco's modified Eagle's medium (DMEM) and centrifuged for 8 minutes at 1000rpm. Supernatant was discarded and cells were re-suspended in 1ml fresh media and were added to a tissue culture treated T25 flask having 3 ml DMEM. For attachment and proliferation flask was placed in incubator at 37°C with 5% CO_2.

Method for the Preparation and Optimization of the Serially Diluted Drugs: For stock preparation, sesamol was dissolved in 100% ethanol and then different working concentrations were made of each drug i.e. sesamol, sesamol loaded liposomal nanocarriers and 5FU and after optimization at IC50 concentration (concentration of drug needed to inhibit 50% of cells). The optimized concentration of drugs at which wound healing assay was performed was 234.72µg/ml for 5-FU, 112.63µg/ml for sesamol, and 86.56µg/ml for sesamol loaded liposomal nanocarriers.

Grouping of the Treated Cells: For treating the cells with different drugs, different groups were made

- Group 1: Control group (untreated HT-29 cells);
- Group 2: 5-FU treated (IC50=234.72µg/ml)
- Group 3: Sesamol treated (IC50=112.63µg/ml)
- Group 4: Sesamol loaded liposomal nanocarriers treated (IC50=86.56µg/ml)

Cell Counting: Cell counting was done using Neubauer counting chamber, cells were observed under an inverted microscope and trypsinized after that 10ul of the cell suspension was put on the chamber for counting. Cells were counted in 4 squares and then average was taken and following formula was used to derive the final cell count: = "2 x no of cells in a chamber $x10^4$"

Cell Migration Assay/ Scratch Assay: 72000 cells were seeded in each well in a 6 well plate. The plate was placed in incubator so that they become 100% confluent and a monolayer formed. A vertical scratch was induced using sterile 10µL tip in each well and cells were treated with the compounds. Images were taken at 0 hour and at 24, 48, 72 and 96 hours to observe wound closure.

Area of wound closure and cellular migration was calculated by using the following formula

"% of wound closure = [(At = 0h−At = h) / At = 0h] × 100%"

Where: At=0h is the area of the wound measured immediately after scratching;

At=Δh is the area of wound measured 0, 24, 48, 72 or 96 hours

Statistical Analysis: SPSS version 24 was used to analyze the data. Numerical data has been presented as mean ± SE of mean (SEM), ANOVA (analysis of variance) was applied to generate this and for intergroup comparison Tukey's post hoc tests was applied. P-value < 0.05 was considered statistically significant between and within the treatment groups.

RESULTS

The Anti-migratory Potential of Untreated and Treated Groups at Different Time Intervals on HT-29 Cell line: To check the anti-migratory potential of treated groups (5-FU, sesamol, sesamol loaded liposomal nanocarriers) in vitro scratch assay was performed and at different time intervals i.e. 0, 24, 48, 72 and 96 hours they were observed under the inverted microscope.

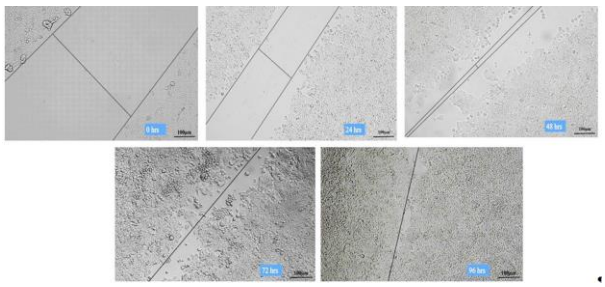

Figure No. 1: Untreated/Control HT-29 group images taken at 10x magnification after different time intervals

In our results the control cells showed 50.27% wound closure at 24 hrs, 94.32% at 48hrs and 100% at 72 hrs (Fig 1, 5), showing the efficient migration potential of these cells. As compared to control group, 5-FU (at IC50) treated cells paused the migration for up to 48 hrs

with highly significant p-value < 0.0001 and complete cell death was observed at 72 hrs (Fig 2, 5).

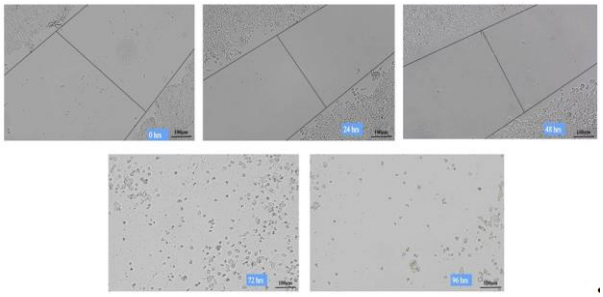

Figure No. 2: Effects of 5-FU treatment on migration of HT-29 cell line

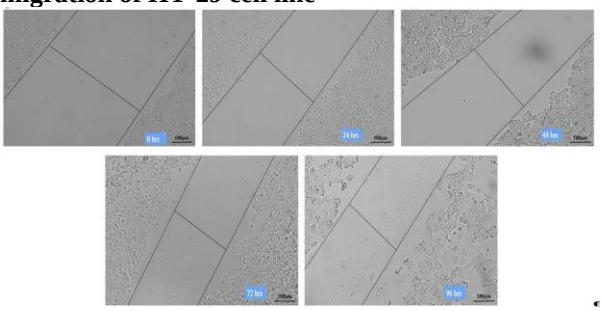

Figure No. 3: Effects of Sesamol treatment on healing/migration of HT-29 cell line

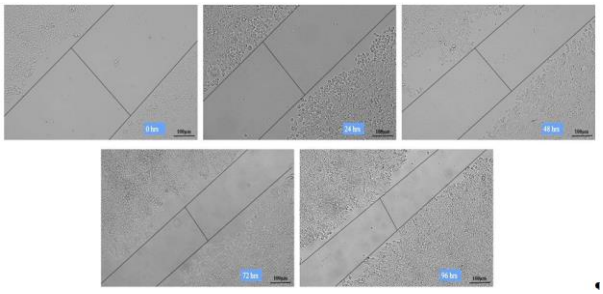

Figure No. 4: Effects of Sesamol loaded liposomal nano-carriers treatment on migration of HT-29 cell line

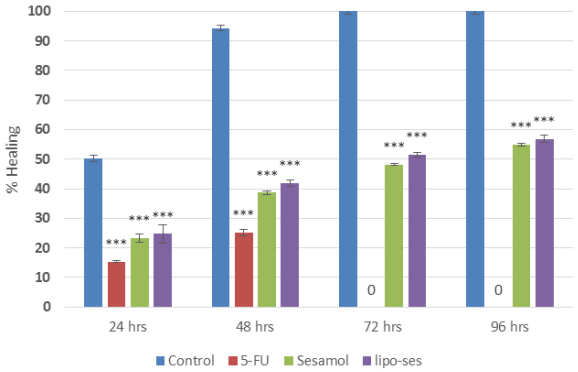

Figure No. 5: Graphical representation of anti-migratory effects of 5-FU, sesamol and sesamol loaded liposomal nanocarrierss on HT-29 cell line

(*** =very highly significant)

(Experiments were run in triplicates, data represented as mean ± SEM)

While sesamol (at IC50) significantly hindered migration i.e, 23.3% at 24 hrs, 38.63% at 48 hrs, 48.19% at 72 hrs, and 54.89 % at 96 hrs as compared to control group (p-value < 0.0001). Sesamol loaded liposomal nano carriers (at IC50)) significantly inhibited the wound closure up to 24.7% at 24 hrs, 41.86 % at 48 hrs, 51.45 % at 72 hrs and 56.87 % at 96 hrs as compared to control group (p-value < 0.0001) (Fig 3,4,5)

DISCUSSION

Wound healing / scratch assay is one of the most primitive and cost-effective technique to study migration of cells in many physiological and pathological conditions such as wound repair and cancer metastasis. In this method an artificial wound is made by scratching the cells with pipette tip on surface of the monolayer of cells. Afterwards images are taken at regular intervals, which are later compared to calculate the percentages of cell migration[11]. Important advantage of this simple technique is that it mimics the in vivo migration of cells to some extent[12]

In our study scratch assay was performed to assess the anti-migratory potential of different compounds i.e., 5-FU, sesamol, and sesamol loaded liposomal nan-carriers (at the IC50 concentrations) .The migration of the cells were observed at different time intervals (0, 24, 48, 72 and 96 hours), and we found that 5-FU showed very excellent anti-migratory effects until 48 hrs with a P value of < 0.0001 that is very highly significant. However, at 72 and 96 hours the cells were completely dead in 5-FU treated group, while sesamol and sesamol loaded liposomal nancarriers inhibited cell migration till 96 hours with a very highly significant P value of < 0.0001. When sesamol and sesamol loaded liposomal nancarriers were compared, delayed in healing% was more prominent in sesamol group indicating that sesamol has greater anti-migratory capacity to halt the migration or proliferation of cancer cells compared to sesamol loaded liposomal nanocarriers and even 5-FU, demonstrating its potential role to stop or delay the metastasis of HT-29 cell line.

Sesamol a phenolic compound obtained from sesame seeds, has a well-established role as chemo preventive, antioxidant, anti-hepatotoxic, anti-mutagenic, anti-inflammatory and anti-aging agent[13,14]. In this regard one study carried out on triple negative breast cancer in vivo and in vitro models, showed that sesamol can significantly diminish the metastatic and proliferative potential of Hs-578T and f MDA-MB-231 TNBC cell lines[15]. One more study was done on lung fibroblasts i.e. DHLF, NHLF and A549 cell lines in which anti-migratory potential of sesamol was observed where the cells treated with sesamol showed significantly reduction in the TGF-β mediated cellular migration[16]. Another study performed on MCF-7 breast cancer cell line, the researchers concluded that sesamol may play

significant roles in remodeling of extracellular matrix (ECM), metabolism of fatty acid and regulation of cell cycle[17]. Though we didn't evaluate the mechanism underlying, various studies confirm that sesamol has shown its anti-migratory effects through alteration in multiple pathways viz. MAPK, JNK, PI3K/AKT, TNFα, and NF-κB pathways[18, 19].

Our results revealed that sesamol has significant anti-migratory effect in its native form as compared to its liposomal nanocarriers which is different from other studies. In a study conducted by Zhu WT et al in 2023 resveratrol liposomal conjugations inhibited cell migration more considerably than their native form[20] One more study stated that in H1975 non-small cell lung cancer cell line, liposomal encapsulation of osimertinib (OB), boosts the efficacy of native OB in hindering the migration of H1975 cells[21]. Further detailed studies are warranted to confirm these findings and to evaluate the precise cellular mechanisms of sesamol for its anti-intrusive and anti-metastatic potential.

CONCLUSION

The current study shows that natural phenolic compound sesamol and its liposomal nanocariers has significant anti-migratory effects on colorectal cell line (HT-29). Further clinical trials will be requisite to prove it as promising agent in treatment of metastatic CRC.

Author's Contribution:

Concept & Design of Study:	Amreen Liaquat
Drafting:	Kauser Ismail, Shumaila Usman
Data Analysis:	Jasra Gul, Raza Shah, Muhammad Owais Ismail
Revisiting Critically:	Amreen Liaquat, Kauser Ismail
Final Approval of version:	Amreen Liaquat

Conflict of Interest: The study has no conflict of interest to declare by any author.

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