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Lycopene: A promising adjunct in the management of cervical cancer as revealed by its antiproliferative and apoptotic effects in HeLa cell lines

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Abstract

Cervical cancer is the second most common cancer globally among females, and is known to be the leading cause of mortality among women worldwide. In the present study, the role of lycopene – a natural antioxidant rich in tomatoes, was investigated in the chemoprevention of cervical cancer. Human cervical cancer cell line (HeLa) was co-cultured with varying doses (0 – 100 µg/ml) of natural antioxidant lycopene for varying time periods (i. e. 0 hr, 24 hr and 48 hr). The anti-proliferative effect of lycopene on HeLa cells was determined by MTT assay, whereas the lycopene-induced apoptosis in HeLa cells were studied by employing ELISA based Caspase-3 Assay. Our results showed an appreciable induction of caspase-3 activity in HeLa cells after 48 hour of treatment at doses of lycopene that were around 70 µg/ml, while it failed to induce apoptosis in 24 hr cultures of HeLa cells. Our data is suggestive that Lycopene may act as a safe

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and economical adjunct in the management of cervical cancer by providing new anticancer adjunct with appreciable efficacy and minimum adverse effects.

Key words: Lycopene, cervical cancer, HeLa cells, apoptosis, caspase-3, anti-proliferation

INTRODUCTION

Globally, cervical cancer (CC) falls within the zone of top cancers in terms of both incidence and mortality, and has become one of the leading causes of cancer deaths amongst women (Yapeng *et al,* 2012), where it accounts for more than 260,000 deaths annually (Senapathy et al, 2011). A number of natural antioxidants are being investigated worldwide by inducing programmed cell death to study the antiproliferative activities of the compound in cancer cells (Mareike Kelkel *et al*, 2011). Lycopene, the natural antioxidant employed in the present study, is one of the members of the carotenoid family, richly present in tomatoes. It is the major carotenoid of plasma and other body tissues (John Sh *et al*, 2004). Lycopene can neutralize free radicals generated from oxygen, where studies have shown that dietary intake of tomatoes and tomato products containing lycopene may reduce the risk of chronic diseases such as cardiovascular diseases and cancer (Giovannucci E, 1999).

Induction of apoptosis in cervical cancer cells by means of natural antioxidants is one of the aspects in the management of cancer and is being studied worldwide. Therefore, in the present study, lycopene has been used to probe that whether it induces apoptosis or not in cervical HeLa cell lines, as it is known for its antioxidant and antiproliferative properties. Our previous study was on cervical cancer Caski cell lines, which had showed promising results. The chemical structure of lycopene is depicted below.

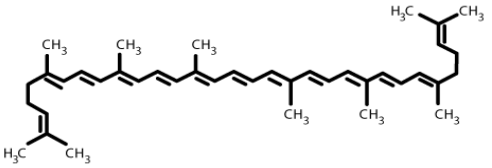

Figure1: Chemical structure of Lycopene ($C_{40}H_{56}$) [Source: Google)

MATERIALS AND METHODS

2.1. Chemicals and reagents.

Commercially available Lycopene, was employed in the present study. MTT, trypan blue, propidium iodide (PI) and Dimethyl sulfoxide (DMSO), were of Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) (v/v), Dulbecco's Modified Eagle's Medium (DMEM), 100 U/ml penicillin and 100 U/ml streptomycin were from Gibco Life Technologies (Carlsbad, CA, USA). HeLa cell line was obtained from National Centre for Cell Science (NCCS) Pune, India. Colorimetric kit of Caspase was of R&D Systems Inc., Minneapolis, MN, USA, while Caspase-3 inhibitor (z-DEVD-FMK) and Caspase-9 inhibitor (z-LEHD-FMK) was of BD PharMingen (San Diego, CA, USA).

2.2. Cell culture.

Human cervical carcinoma cell lines (HeLa) were cultured in DMEM medium (supplemented with 10% FBS, 2 mM glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin) in 96-well plates at 37°C with 5% CO_2 and air humidity of 95% as described by us previously (Imran et al., 2018). Exponentially, growing cells were used for experiments. $5x10^6$ cells (HeLa) in 75-T flasks were treated with or without Lycopene (0-100 ug/ml) and were cultured for 24 hrs and 48 hrs respectively. Thereafter, the cells were harvested and processed as per experimental design.

2.3. MTT assay.

The cytotoxic effect of Lycopene (0-100 ug/ml) on cervical cancer HeLa cells was determined by MTT assay as described by us earlier (Hasan et al, 2006 and 2007). Exponentially growing HeLa cell lines ($5x10^4$

/well) were seeded onto 96-well culture plate and allowed to attach overnight, followed by treatment with various doses of lycopene (0-100 µg/ml) for 24 h. 10µl MTT (5 mg/ml) was added to each well and incubated for another 4 h at 37°C. Dimethyl sulfoxide (DMSO) was added to each well and thoroughly mixed to dissolve the purple crystals. The absorbance of each well was measured on a microplate reader, using a wavelength of 570 nm and a reference wavelength of 650 nm and the cell survival was expressed as the percentage (%) over the untreated control.

2.4. Assay of Caspase-3 Activity.

HeLa cells ($5x10^6$ cells) in 75-T flasks were treated with or without Lycopene (0-100 ug/ml), then incubated for 24 hr and 48 hr to detect the activity of caspase -3 and assessed according to manufacturer's instruction of the Caspase colorimetric kit (R&D Systems Inc., Minneapolis, MN, USA). Cells were collected and lysed in 50 µl lysis buffer containing 2 mM DTT, for 10 min. After centrifugation, the supernatant containing 200 µg protein were hatched with caspase - 3 substrates in reaction buffer. Then all samples were incubated in a 96 well flat bottom microplate at 37˚C for 1 h. Levels of discharged pNA were measured with ELISA reader (Anthos Labtec Instruments GmbH, Salzburg, Austria) at 405 nm wavelength (Hsiao et al, 2012; Imran et al, 2018).

2.5. Statistical Analysis.

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett test, by considering $p<0.05$ as significant.

RESULTS

3.1. Anti-proliferative effect of Lycopene on HeLa cells.

Confluent cervical cancer HeLa cells were co-cultured with varying doses of lycopene (0-100 ug/ml) for 24 hr and 48 hr respectively, and in turn, were subjected to MTT assay as described in methods. As revealed by MTT assay, none of the doses of lycopene showed any significant effect on the proliferation of HeLa cells (Fig. 2). On the other hand, interestingly, increase in time period of co-culturing with

lycopene exhibited a significant anti-proliferative effect. As evident from Fig. 2, it was observed that a dose of around 70 µg/ml of lycopene significantly inhibited the proliferation of human cervical cancer cells, in a dose-dependent manner in 48 hr cultures.

3.2. Caspase-3 activity assay.

As a matter of fact, the downstream signals during apoptosis are transmitted via caspases which, upon conversion from pro to active forms, mediate the cleavage of Poly (ADP-Ribose) Polymerase (PARP). So, we then investigated the possible role of caspase-3 in inducing apoptosis of HeLa cells by Lycopene.

Hence, HeLa cells were treated with different doses of lycopene (0-100 µg/ml) for 24 h and 48 h. Cytosolic proteins were extracted and assayed for caspase activity by incubation with a chromogenic substrate, DEVD-pNA (for caspase-3). Our results showed an appreciable induction of caspase-3 activity in HeLa cells after 48 h of treatment at doses of lycopene above 50 µg/ml (Fig. 3) Insignificant caspase-3 activity was observed in HeLa cells after 24 h of treatment (Fig. 3). The caspase-3 activity was observed to be dose and time dependent and maximal at the dose of 70 µg/ml after 48 h of treatment with lycopene. Furthermore, a well established and known anti-proliferative compound, mainly N-Acetyl Cysteine (NAC) (10 mM) was also employed in the present study as a positive control. As evident from data depicted in Table 1, our data are in accordance to our previous studies (Imran et al, 2018), where 10 mM of NAC showed high activity levels of caspase-3 in cervical cancer cell lines.

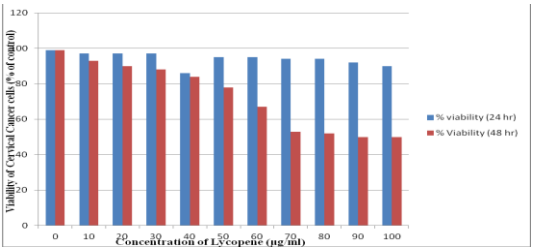

Figure 2: Dose response and time dependent anti-proliferation assay: MTT cell viability assay in HeLa cells after 24 hour and 48 hour of treatment with Lycopene (0-100 ug/ml) (n=20; p<0.001)

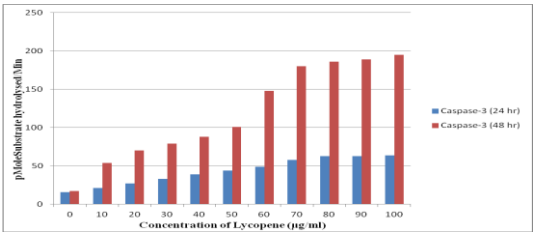

Figure 3: Dose and time dependent Caspase-3 activity assay in HeLa cells after 24 hour and 48 hour of treatment with Lycopene (0-100 ug/ml) (n=20; p<0.001)).

Table 1: Caspase 3 activity assay in untreated and NAC (10 mM) treated HeLa Cell Lines

Untreated HeLa Cell Lines (Controls)	**Caspase-3 activity** (pMol Substrate Hydrolyzed/Minute)	**NAC (10 mM) + HeLa Cell Lines**	**Caspase-3 activity** (pMol Substrate Hydrolyzed/Minute)
Control Sample 1	3.78	Test sample 1	120.05
Control Sample 2	5.02	Test sample 2	131.76
Control Sample 3	6.27	Test sample 3	123.45
Control Sample 4	9.23	Test sample 4	109.34
Control Sample 5	6.12	Test sample 5	115.38
Control Sample 6	8.28	Test sample 6	119.32
Control Sample 7	8.22	Test sample 7	129.45
Control Sample 8	9.12	Test sample 8	120.02
Control Sample 9	10.28	Test sample 9	137.28
Control Sample 10	7.29	Test sample 10	118.72

Table 1: Caspase-3 activity assay with NAC: N-Acetyl Cysteine (NAC) (10 mM) treated HeLa cell lines (n=10 each for control and test samples) were cultured for 24 hrs. It exhibited high activity levels of caspase-3, whereas untreated control cells (n=10) showed negligible / insignificant caspase-3 levels. Caspase-3 activity assay was carried out as described in methods, and that, the values displayed are the means of 10 independent experiments each (control and test samples respectively), carried out in duplicates for casapase-3 ($p<0.001$ for all).

DISCUSSION

Cervical cancer is one of the most common gynecological cancer and one of the leading causes of cancer deaths amongst women. There are nearly 400,000 new cases of cervical cancer diagnosed every year globally and 80% of these are diagnosed in the developing countries (Rajendra *et al*. 2006). The fundamental basis of carcinogenesis is the

imbalance between apoptosis and the rate of proliferation of the tissue cells. Therefore, induction of apoptosis in the tumor cells may prove to be helpful in the treatment of malignancies (Shaista *et al*. 2012). Lycopene is one of the most potent natural antioxidants among dietary carotenoids. It is responsible for its remarkable chemopreventive and antiproliferative activity. Lycopene prevents the promotion of carcinogenesis as it interferes with various cellular processes including cell cycle progression and the modulation of signal transduction pathways. Studies have showed that lower concentration of lycopene only affects the lipid peroxidation, whereas its higher concentrations causes arrested G_2/M phase by inducing DNA damage, eventually leading to cell cycle arrest followed by apoptosis (Mareike Kelkel *et al*, 2011). Earlier studies have shown that an increase in dietary consumption of lycopene was associated with decreased prostate cancer development in a case-control study (Pourmand G, *et al*, 2007). According to the previous findings of (Asmah *et al*. 2002), lycopene was also found to show its antiproliferative activity in human breast and liver cancer cell lines as well as effect on the cell cycle and viability in HT-29 (Human Colorectal Adenocarcinoma Cell line), T-84 (Human Colorectal carcinoma cell line) and MCF-7 (human breast cancer cell line) (Teodoro *et al*. 2012).

In the present study, we made an attempt to probe the effects of lycopene on the cell viability and induction of apoptosis in HeLa cell line. The data of MTT cell viability assay and Caspase-3 assay by co-culturing the HeLa cell with varying doses of natural antioxidant lycopene (0 – 100 µg/ml) for two different time periods of 24 hr and 48 hr showed appreciable degree of induction of apoptosis and antiproliferative activity towards after 48 h of treatment at doses of lycopene that were around 70 µg/ml. Caspase-3 is an important 'executioner caspase' in programmed cell death. They are synthesized in the cell in its inactive form called as pro-caspases and their activation is mediated by the cleavage of aspartic acids by other 'initiator caspases' such as caspase-8 and caspase-9 (Park K, et al, 2009). Caspase-3 cleaves cellular substrates and therefore is a crucial mediator in apoptotic cell death (Kaufmann, S.H. *et al*, 2000). Activation of the caspase-3 pathway is a hallmark of apoptosis and can be assayed in order to study the apoptotic status of the cell.

Our preliminary data is indicative for the lycopene induced antiproliferation and induction of apoptosis in cervical cancer HeLa cell lines, and hence, may provide a direction for future in-depth studies for identifying a safe, economical adjunct cervical cancer management.

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REFERENCES

1. Anderson Junger Teodoro, Felipe Leite Oliveira, Nathalia Balthazar Martins, Guilherme de Azevedo Maia, Renata Brum Martucci and Radovan Borojevic. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. Cancer Cell International 2012, 12:36
2. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J National Cancer Institute,* 91:317 (1999)
3. Hasan, N., Siddiqui, M. U., Toossi, Z., Khan, S., Iqbal, J. and Islam, N. Allicin-induced suppression of Mycobacterium tuberculosis 85B mRNA in human monocytes. Biochem. Biophys. Res. Comm., 2007; 355(2): 471476. 36.
4. Hasan, N., Yusuf, N., Toossi, Z. and Islam, N., Suppression of Mycobacterium tuberculosis induced reactive oxygen species (ROS) and TNF- α mRNA expression in human monocytes by allicin. FEBS Letters, 2006; 580: 2517-2522.
5. Hsiao YP, Yu CS, Yu CC, Yang JS, Chiang JH, Lu CC . Triggering apoptotic death of human malignant melanoma A375. S2 cells by bufalin: Involvement of caspase cascade-dependent and independent mitochondrial signaling pathways. Evid Based Complement. Alternat. Med. (2012) 591241.
6. Imran, M. A., Mahdi, A. A. and Islam, N., Natural Phytochemicals Induces Apoptosis in Cervical Cancer Cell

Line: A Comparative Preliminary Study, Eur. Acad. Res., 2018; 6(1): 42-58.

7. J Giftson Senapathy et al, The present scenario of cervical cancer control and HPV epidemiology in India: an outline. Asian Pacific Journal of Cancer Prevention, Vol 12, 2011.
8. John Sh, Qin Qu, Yukio Kakuda, David Yeung, Yueming Jiang. Stability and Synergistic Effect of Antioxidative Properties of Lycopene and Other Active Components. *Critical Reviews in Food Science and Nutrition*, 44:559–573 (2004)
9. Kaufmann, S.H. Earnshaw, W.C. Induction of apoptosis by cancer chemotherapy.Exp Cell Res **256**, 42–9 (2000).
10. Mareike Kelkel , Marc Schumacher , Mario Dicato & Marc Diederich. Antioxidant and anti-proliferative properties of lycopene. *Free Radical Research,* August 2011; 45(8): 925–940
11. Park K, Yi SY, Kim UL, et al. Monitoring of cleavage preference for caspase-3 using recombinant protein substrates. J Microbiol Biotechnol. 2009; 19(9):911–917.
12. Pourmand G, Salem S, Mehrsai A, Lotfi M, Amirzargar MA, Mazdak H, Roshani A, Kheirollahi A, Kalantar E, Baradaran N, et al. The risk factors of prostate cancer: a multicentric case-control study in Iran. Asian Pac J Cancer Prev 2007; 8:422–8.
13. Rajendra A Kerkar, Yogesh V Kulkarni. Screening for cervical cancer : an overview. J Obstet Gynecol India Vol. 56, No. 2: March/April 2006; 115-122.
14. Shaista M. Vasenwala, Reema Seth, Nazima Haider, Najmul Islam, Tamkin Khan,Veena Maheshwari, Suhail ur Rehman. A study on antioxidant and apoptotic effect of Azadirachta Indica (neem) in cases of cervical cancer. Gynaecologic oncology 2012.
15. Yapeng Hu, Liwu Fu. Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res 2012; 2(3):340-356.