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IN-VITRO ANTI-PROLIFERATIVE ACTIVITIES OF LYCOPENE AGAINST PROSTRATE, LUNG, COLON AND BREAST CANCER CELL LINES

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ABSTRACT

Lycopene crystals were obtained by solvent extraction of tomato peel isolated from processing waste followed by crystallisation. Microscopic, spectroscopic and HPLC confirmed nearly 92% purity of lycopene crystals. The effectiveness of lycopene crystal from waste, standard lycopene and β-carotene (10-100 μM) was evaluated for cell growth inhibition of prostrate (PC-3 and DU-145), colon (HCT-15, HT-29, & 502713), lung (A549, HOP62) and breast (MCF-7) cancer cell lines. Standard lycopene and lycopene crystals showed >95 % cell growth inhibition towards prostate and lung cancer cell lines, however β-carotene did not proved effective inhibitor against these cancer cell lines. Increase in growth inhibition with increasing carotenoids concentration was observed for all cell types under investigation. Results on colon cancer cell lines revealed that the standard lycopene was more effective than lycopene crystals and standard β-carotene. However, β-carotene showed higher growth inhibition for breast cancer cell lines than lycopene crystals and standard lycopene.

Keywords: Tomato, lycopene, β-carotene, anti- proliferative, cancer cell lines

INTRODUCTION

The agricultural industry waste proves to be an immense reservoir of materials of natural origin. By-products of fruits and vegetables processing represent a major disposal problem, but they are also promising sources of compounds which may be used for various purposes in the food, pharmaceutical and cosmetic industries. Processing of tomato into juice, ketchup, paste, puree etc. lead to the generation of huge amount of waste. Tomato processing industry produces a considerable amount of waste and its exploitation for the extraction of carotenoids (lycopene, lycopene-5,6-diol, lutein, β-carotenes, neurosporene, phytoene, and phytofluene) is an promising field due to their health promoting effects (George et al., 2004; Hazara et. al., 2014). Lycopene, represents more than 80% of the total tomato carotenoids and responsible for the characteristic deep-red color (Nguyen and Schwartz, 1999). It is an effective antioxidant with eleven conjugated double bonds having high singlet oxygen quenching ability compared other carotenes (Di Masico et. al., 1989). Lycopene, one of the most extensively studied carotenoids in tomatoes, possesses potent anti-oxidant activity due to its conjugated hydrocarbon chain (Li et al., 2011; Van Breemen and Pajkovic, 2008).

Epidemiological studies supports that intake of lycopene provides protection against several types of cancer cells like prostate, colon, breast, lung, endometrium and epithelial cancers (Levy et. al., 1995; Sengupta et. al., 2003; Murtaugh et. al., 2004; Sarkar et. al., 2010). It has been reported that lycopene synergizes with other natural compounds, such α-tocopherol and 1,25 dihydroxyvitamin D3, in inhibiting prostate carcinoma cell proliferation (Pastori et. al., 1998), HL-60 leukemic cell differentiation (Amir et. al., 1999), and low-density lipoprotein (LDL) oxidation (Fuhrman et al., 2000).

Lycopene has been suggested to prevent carcinogenesis and atherogenesis by protecting critical bio-molecules such as lipids, low-density lipoproteins (LDL), proteins and DNA (Pool-Zobel et. al., 1997; Rao and Agarwal, 1998). Antioxidant property of lycopene has inhibitory effect on DNA synthesis, initiate up-regulation of gap-junction proteins, synthesis of cytoprotective enzymes and a reduction of local androgen signalling, impact IGIF-1 signalling, antioxidant activity and induction of apoptosis cell death, signifying that carotenoids are promising chemopreventative agents with

genomic and non-genomic cellular effects (Zhang et. al., 1992; Wertz et. al., 2004; Kumar et al., 2008; Richard and Van Breemen, 2008).

Present study has been undertaken to study the effect of lycopene crystals on anticancer properties along with standard lycopene and β-carotene.

MATERIALS AND METHODS

TOMATO WASTE

Tomato pomace was collected from a tomato paste manufacturing unit located in Amritsar, India during the processing season. Tomato skin was separated from pomace using a flotation-cum-sedimentation system (Kaur et. al., 2005) and used for preparation of lycopene crystals.

CHEMICALS AND CANCER CELL LINES

Standard lycopene & β-carotene were obtained from Sigma Aldrich, USA. Cancer cell lines of prostrate (DU-145 and PC-3), lung (A549, HOP62), colon (HCT-15, HT-29 and 502713), and breast (MCF-7) were obtained from Pharmacology Division, Indian Institute of Integrative Medicines, Jammu, India.

PREPARATION OF LYCOPENE CRYSTALS

Tomato carotenoids were extracted from the tomato processing waste peel with ethyl acetate. Extracts were combined and then evaporated in vacuum evaporator (Buchi, USA). The extracted oleoresin containing lycopene crystals was saponified using alkaline propylene glycol solution (oleoresin: propylene glycol: alkali: water: 5:3:1:1) at 65 ^{o}C for 2 h. Finally the water was added to disperse the impurities and filtered through Whatman No. 4 filter paper. The extracted lycopene crystals were dried in freeze drier (Heto, Denmark) and kept at -20^{o}C (Ausich and Sanders, 1999; Hartal et. al., 1999).

MICROSCOPY OF LYCOPENE CRYSTALS

Lycopene crystals were mounted on a slide in immersion oil, covered with cover slip and observed under light microscope (Olympus, Tokyo, Japan). The observed images under the light microscope were captured by digital camera (C-5060, Olympus, Tokyo, Japan).

HPLC ANALYSIS

Lycopene extract and crystals from tomato waste skin along with standard lycopene and β-carotene solutions were prepared in hexane and filtered through 0.22 µm filter (Milli-pore, Billerica, USA). Samples were analyzed on analytical HPLC fitted with an automatic degasser, C-18 column 4.6×250 mm and Photo-diode array detector (PDA) (Waters, Milford, USA). A mobile phase of acetonitrile: dichloromethane: methanol (45:10:45 v/v) was degassed by a supersonic bath and filtered through a 0.22 mm filter paper. The detector was set in scan mode, 210-550 nm during the analysis. The column temperature was maintained at 25 ^{o}C while flow rate was set at 1 ml/min. A sample volume of 30 µl was injected into the column. The carotenoid content of extract and crystals obtained from tomato waste skin were measured by comparing peak retention time and area under the chromatographic peak of standard lycopene and β-carotene (Emenhiser *et. al.*, 1995).

UV SPECTRUM OF LYCOPENE CRYSTALS

Lycopene crystals extracted from tomato waste skin and the standard lycopene were dissolved in hexane (1mg/ml) and scanned over the wavelength of 200-800 nm in UV visible spectrophotometer (Shimadzu Co Ltd, Japan).

ANTICANCER ACTIVITY OF LYCOPENE CRYSTALS

Cells were grown in tissue culture flasks containing complete growth medium (RPMI-1640 medium with 2 mM glutamine, 100 µg/ml streptomycin, pH 7.4, sterilized by filtration and supplemented with 10% fetal calf serum and 100 units/ml penicillin before use) at 37 ºC in an atmosphere of 5% CO_2 and 90% relative humidity in a carbon dioxide incubator. The cells at sub-confluent stage were harvested from the flask by treatment with trypsin 0.05% in phosphate buffer saline (PBS) containing 0.02% EDTA. Cells with viability of more than 98%, as determined by trypan blue exclusion, were used for assay. The cell suspension of $1x10^5$ cells/ml was prepared in complete growth medium for determination of cytotoxicity.

Stock solutions of 0.02 M of all the samples were prepared in DMSO and THF for different experiments. The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain 10-100 µM solutions.

In vitro cytotoxicity against human cancer cell lines was determined following Monks et al., (1991) by three different experiments, using 96-well tissue culture plates. The 100 µl of cell suspension was added to each well of the 96-well tissue culture plate. The cells were incubated for 24 h. Test materials in complete growth medium (100 µl) were added after 24 h incubation to the wells containing cell suspension. The plates were further incubated for 48 h (at 37^{o}C in an atmosphere of 5% CO_2 and 90% relative humidity in a carbon dioxide incubator) after addition of test material and then the cell growth was stopped by gently layering trichloroacetic acid (50% TCA, 50µl) on top of the medium in all the wells. The plates were incubated at 4^{o}C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove TCA, low molecular weight metabolites, serum proteins etc. and air-dried. Cell growth was measured by staining with Sulforhodamine B dye (Skehan et. al., 1990). The adsorbed dye was dissolved in Tris-Buffer (100 µl, 0.01M, pH 10.4) and plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA (Enzyme Linked Immunosorbent Assays) reader at 540 nm.

The cell growth was calculated by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in

absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated.

STATISTICAL ANALYSIS

The values are represented as mean value of three replicates. Analysis of variance (ANOVA) was performed to evaluate the difference between the different replicates. All the analysis differences were carried out at 95 % level of confidence.

RESULTS AND DISCUSSION

ANALYSIS OF LYCOPENE CRYSTALS

The structure of the lycopene crystal observed under light microscope is presented in Fig. 1. Microscopic images showed bright red coloured needle like structure. Previous studies have also reported similar structure of the lycopene crystals (Nunes and Mercadante, 2004).

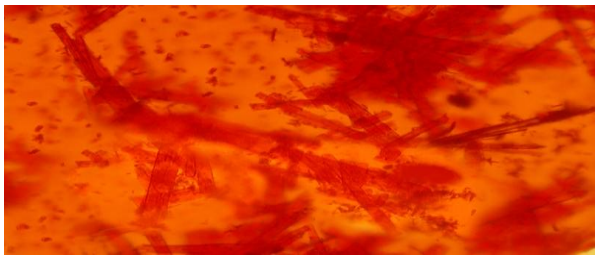


Fig. 1- Needle like crystals of lycopene

The HPLC spectra of crude lycopene extract, crystals and standard lycopene and β-carotene are presented in Fig. 2. The results revealed that solvent extract and lycopene crystals contained both lycopene and β-carotene. However, the relative concentration of β-carotene was higher in extract but it reduced in crystals. It might be due to the loss of β-carotene during purification process. Further analysis revealed that lycopene crystals prepared from tomato waste skin contained 92.68 % lycopene and 7.23 % β-carotene. HPLC chromatogram did not show the presence of other impurities. Previous studies have reported 95 % purity for lycopene crystals prepared from tomato paste (Yaping et. al., 2002) and 90 % purity in case of crystals from tomato skin (Ausich and Sanders, 1999).

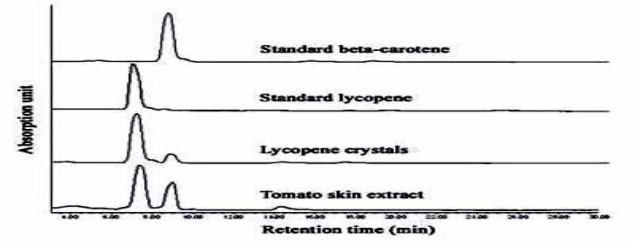


Fig. 2-HPLC chromatogram of tomato skin extract, lycopene crystals, standard lycopene, standard β-carotene using C-18 column eluted with acetonitrile: dichloromethane:methanol (45:10:45) as mobile phase

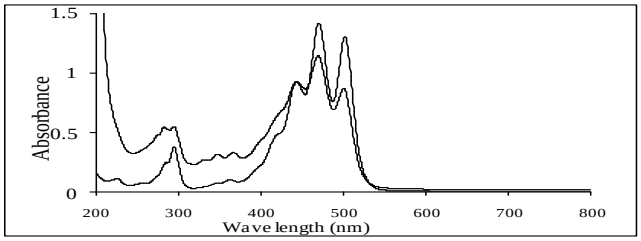


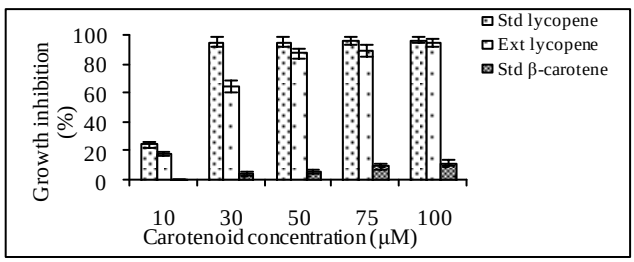
Fig. 3. The scanning pattern bands of standard lycopene and lycopene crystals

The purity of lycopene was further established by UV spectrum of the lycopene crystals along with standard one. The spectrums for standard and lycopene crystal over the wavelength of 200-800 nm were similar (Fig. 3). The major three bands of both the samples coincided at 441.01, 468.5 and 503.5 nm. Earlier research also showed that major three bands lies in 448-448.2, 471.6-473 and 503.2-504 nm (Bindl et. al., 1970; Sharma and Maguer, 1996). Thus present results find support from earlier studies.

ANTI PROLIFERATIVE ACTIVITY OF LYCOPENE AGAINST HUMAN CANCER CELL LINES

PROSTATE CANCER

Lycopene caused a significant modification of cell viability in prostate cell lines. Studies on the effect of carotenoids on the anticancer activities against prostate cancer cell lines (DU-145) revealed that standard lycopene inhibited the growth of cancer cell to an extent of 30% at a concentration of 10 µm and further increase in concentration, inhibited the cell growth completely (Fig. 4A). The extracted lycopene crystals were comparatively less efficient in inhibiting the growth of prostate cell line (DU-145) than standard lycopene. The level of inhibition by lycopene crystals increased gradually from initial value of 20% to nearly complete inhibition at 100 µM concentration. The growth inhibition increased sharply as the concentration increased from 10 to 50 µM and there after it became slow. The growth inhibition by β-carotene in prostate cancer cell line (DU-145) was very low at 10 µM concentration and with further increase in concentration up to 100 µM growth inhibition was increased up to 15%. Similar results for standard and extracted lycopene crystals towards prostrate cancer cell line PC-3 were found at lycopene concentrations of 10-100 µM, but the inhibition of β-carotene was lower than that of prostrate cancer cell line DU-145 (Fig. 4B).



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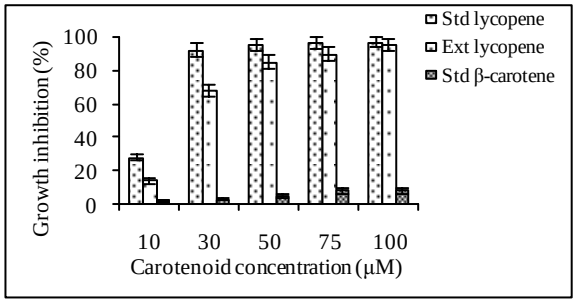

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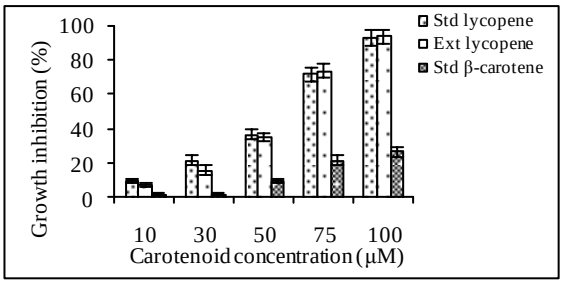

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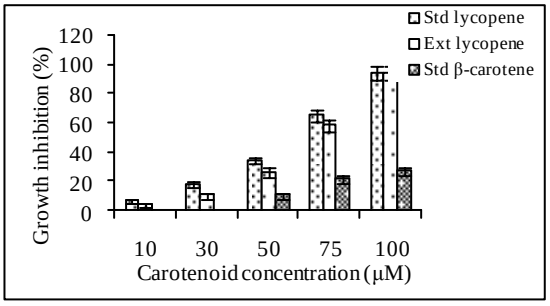


(D)

Fig. 4- Effect of standard lycopene, standard β-carotene and lycopene crystals from tomato skin on growth inhibition (%) of prostate cancer cell line DU-145 (A) and PC-3 (B), lung cancer cell line A549 (C) and HOP62 (D) at 48 h incubation.

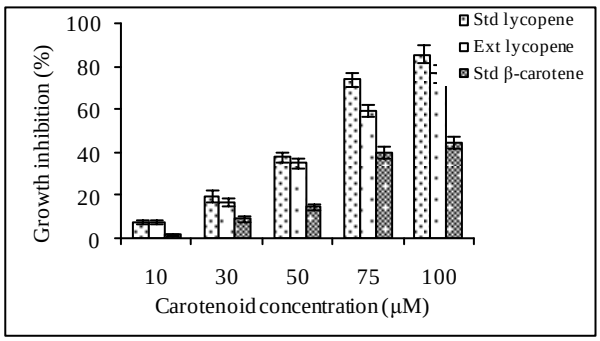
The relationship between lycopene intake and prostate cancer risk has been supported by studies linking low plasma level of lycopene with an increased risk (Giovannucci et. al., 1995). Lycopene has been shown to concentrate in prostate tissues which protect them against free radicals & other harmful metabolites (Vecchia, 2002). Intakes of β-carotene, α-carotene, lutein and β-cryptoxanthin were not significantly associated with a reduction in prostate cancer risk, but high lycopene intake resulted in a statistically significant risk reduction of 21% prostate cancer (Hsing et. al., 1990). Consumption of tomatoes and tomato based products, accounting for 82% estimated lycopene intake, was associated with a 35% reduced risk of total prostate cancer (Giovannucci et al., 1995). Adding lycopene to medium containing the LNCaP human prostate adenocarcinoma cell line resulted in decreased DNA synthesis and inhibition of androgen-receptor gene element activity and expression (Zang et. al., 2010). The apparent protective effect against more advanced prostate cancer which might cause death suggests that lycopene supplementation may be beneficial in preventing the progression of prostate cancer

LUNG CANCER

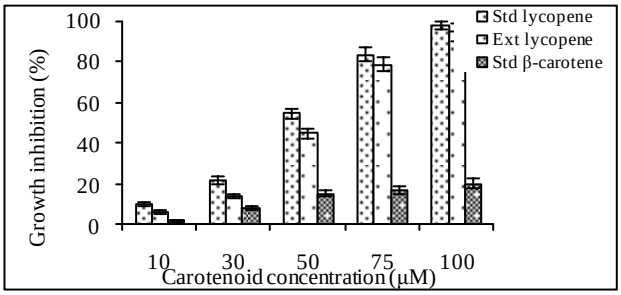
Investigation on cell growth inhibition of lung cancer cell line A549 and HOP62 revealed that as the concentration of standard and extracted lycopene increased from 10 to 100 μM the growth inhibition of both the cancer cell lines increased from about 10 to 94% (Fig 4. C-D). However, β-carotene did not inhibit cell growth like lycopene and exhibited the maximum inhibition up to 27% at 100 μM for both cell types. However the quantum of inhibition by β-carotene was higher in case of lung cancer than prostate cancer cell lines. Administration of lycopene enriched tomato oleoresin had no effect on the development of induced lung tumors in mice (Hecht et. al., 1999). Lycopene inhibited the development of carcinogenesis in the lungs of male mice (Kim et. al., 2000). Studies showed that diet rich in carotenoids, including tomatoes and tomato based products, might reduce the risk of cancer (Holick et. al., 2002).

COLON CANCER

The effect of standard lycopene, extracted lycopene and standard β-carotene on the growth of three colon cancer cell lines has been illustrated in Fig. 5. A-C. Growth inhibition of colon cancer cell lines HT-29, 502713 and HCT-15 increased as the concentration of the three carotenoids increased. The level of inhibition was the maximum for standard lycopene followed by extracted lycopene crystals and standard β-carotene. In case of standard and extracted lycopene crystals the growth inhibition increased sharply with increase in concentration up to 100 μM. The inhibition effect of lycopene was the highest on colon cancer cell line 502713 followed by HCT-15 and HT-29. β-carotene inhibited cell growth of 2-20% for colon cancer cell type 502713, 2-45 % for type HT-29 and 4-59% for type HCT-15 corresponding to the concentration of 10-100 μM. These results indicated that the growth inhibition of β-carotene towards colon cancer lines were higher than prostrate cancer cell lines. Tomato juice rich in lycopene inhibited colon cancer cell growth up to 84% at a concentration of 17 ppm in seven weeks old female rats (Narisawa et. al., 1998). In vitro assays of lycopene against digestive cancers have revealed inhibitory effects on cancer cell growth (Velmurugan *et. al.,* 2002). Lycopene concentration of 10 μM for 24 hour period inhibited the proliferation of colon cancer upto 47% (Tang *et. al.,* 2008).



(A)



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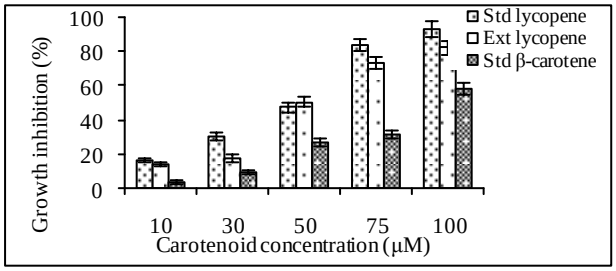

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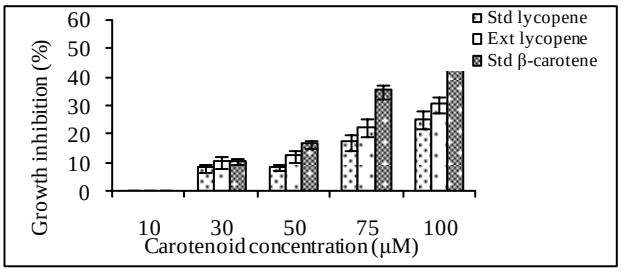

(D)

Fig. 5- Effect of standard lycopene, standard β-carotene and lycopene crystals from tomato skin on growth inhibition (%) of colon cancer cell line HT-29 (A), 502713 (B) and HCT-15 (C), breast cancer cell type MCF-7 (D) at 48 h incubation.

BREAST CANCER

The cell growth inhibition towards breast cancer cell line MCF-7 revealed that the lycopene showed least cell growth inhibition as compared to other cancer cell line of this study (Fig. 5 D). β-carotene showed higher breast cell growth inhibition of 10-47% as compared to standard (8-25%) and extracted (10-39%) lycopene. Previous studies have shown mixed results to the association between lycopene intake and breast cancer. Studies on potential protective effects of carotenoids and antioxidants on breast cancer did not indicate any association with lycopene (London *et. al.*, 1992; Garland *et. al.*, 1993). Recent studies also showed no consistent association for lycopene and breast cancer (Vecchia, 2002; Terry, 2002). However, Nagasawa *et al*. (1995) showed that spontaneous mammary tumors were inhibited in mice fed a lycopene-rich diet. Chalabi et. al., (2007) studied the effects of lycopene on breast cancer cell lines using pangenomic arrays and showed that there is some epidemiologic evidence for a preventive role in breast cancer.

Study revealed that anticancer activity of pure lycopene against prostate, lung and colon cancer was slightly higher than extracted lycopene from tomato waste skin which was due to the presence of 7.23% β-carotene in extracted lycopene crystals. Since the anticancer activity of the β-carotene on the above cancer cell lines was less therefore extracted lycopene showed lower activity. However, in breast cancer, β-carotene showed highest growth inhibition therefore anticancer activity of extracted lycopene crystals was higher than that of standard lycopene.

CONCLUSION

Lycopene crystals prepared from tomato waste skin showed a high degree of purity (92.68%). Lycopene showed strong anticancer activity against prostate followed by lung and colon cancers while breast cancer was better inhibited by β-carotene. The extracted lycopene at 100-μM level was quite effective against prostate, lung and colon cancer. The technique could be exploited to extract bio-active functional ingredient from tomato processing waste which could offset the product cost, solve waste disposal problem and develop nutritional supplement in food products.

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