EVALUATION OF IN VITRO CYTOTOXIC ACTIVITY OF VARIOUS EXTRACTS OF TURMERIC POWDER (CURCUMA LONGA) AGAINST HUMAN PROSTATE CANCER CELL LINE DU-145

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ABSTRACT

DU145 (DU-145) and PC3 human prostate cancer cell lines are the "classical" cell lines of prostatic cancer. DU145 cells have moderate metastatic potential compared to PC3 cells which have high metastatic potential. The DU145 cell line was derived from brain metastasis. DU145 are not hormone-sensitive and do not express prostate-specific antigen (PSA). It has been demonstrated that administration of NFκB ligand RANKL promoted DU145 cell invasion in bone, resulting in osteolytic lesions. DU145 cells also produce soluble factors that activate pre-osteoblast precursors and increase RANKL expression, thus facilitating prostate cancer metastasis in bone. The main objective of the present research work is the evaluation of in vitro cytotoxic activity of various extracts (METP, EETP and CETP) of turmeric powder (Curcuma longa). The in vitro cytotoxic activity was carried out against human prostate cancer cell line DU-145 by SRB assay. The results obtained from the in-vitro studies performed by SRB assay by using human prostate cancer cell line DU-145 displayed that the extracts METP, EETP and CETP possessed a very good cytotoxic activity. From the present studied it had been concluded that METP, EETP and CETP, all were exhibiting the potential cytotoxic action on DU-145 cell line which was proved by using standard drug 5-FU and it was found that METP, with the highest 92.80% cell growth inhibition at 10 µg (IC₅₀ = 2.5 µg/ml), EETP with the 93.83% cell growth inhibition at 10 µg (IC₅₀ = 2.3 µg/ml) and CETP with the 94.52% cell growth inhibition at 10 µg (IC₅₀ = 2.1 µg/ml). The IC₅₀ value of standard drug 5-FU was found to be 1.5 µg/ml with 96.54 % growth inhibition at concentration 50 µg/ml.

KEYWORDS: DU-145, Metastatic potential, Prostate-specific antigen, cytotoxic activity, SRB, assay, IC₅₀ etc.

INTRODUCTION

Turmeric (Curcuma longa)¹¹ is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae.² It is native to southwest India, requiring temperatures between 20 and 30 °C (68 and 86 °F) and a considerable amount of annual rainfall to thrive.²⁶ Plants are gathered annually for their rhizomes and propagated from some of those rhizomes in the following season. When not used fresh, the rhizomes are boiled for about 30-45 minutes and then dried in hot ovens, after which they are ground into a deep-orange-yellow powder²⁷ commonly used as a spice in Bangladeshi cuisine, Indian cuisine, Pakistani cuisine and curries, for dyeing, and to impart color to mustard condiments. One active ingredient is curcumin, which has a distinctly earthy, slightly bitter, slightly hot peppery flavor and a mustardy smell. India, a significant producer of turmeric²⁸ has regional names based on language and state.

Fig 1: Turmeric field in an Indian village
History and etymology
Turmeric has been used in Asia for thousands of years and is a major part of Siddha medicine.[3] It was first used as a dye, and then later for its medicinal properties.[4] The origin of the name is uncertain, possibly deriving from Middle English/early modern English as turmeryte or tarmaret. There was speculation that it may be of Latin origin, terra merita (merited earth).[5] The name of the genus, Curcuma, is from an Arabic name of both saffron and turmeric.

Botanical description
Appearance: Turmeric is a perennial herbaceous plant that reaches up to 1 m tall. Highly branched, yellow to orange, cylindrical, aromatic rhizomes are found. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade.[6] From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm long. The simple leaf blades are usually 76 to 115 cm long and rarely up to 230 cm. They have a width of 38 to 45 cm and are oblong to elliptic, narrowing at the tip.

Inflorescence, flower, and fruit: In China, the flowering time is usually in August. Terminally on the false stem is a 12- to 20-cm-long inflorescence stem containing many flowers. The bracts are light green and ovate to oblong with a blunt upper end with a length of 3 to 5 cm. At the top of the inflorescence, stem bracts are present on which no flowers occur; these are white to green and sometimes tinged reddish-purple and the upper ends are tapered[7]. The hermaphrodite flowers are zygomorphic and threefold. The three 0.8- to 1.2-cm-long sepals are fused, white, have fluffy hairs and the three calyx teeth are unequal. The three bright-yellow petals are fused into a corolla tube up to 3 cm long. The three corolla lobes have a length of 1.0 to 1.5 cm, and are triangular with soft-spiny upper ends. While the average corolla lobe is larger than the two lateral, only the median stamen of the inner circle is fertile. The dust bag is spurred at its base. All other stamens are converted to staminodes. The outer staminodes are shorter than the labellum. The labellum is yellowish, with a yellow ribbon in its center and it is obovate, with a length from 1.2 to 2.0 cm. Three carpels are under a constant, trilobed ovary adherent, which is sparsely hairy. The fruit capsule opens with three compartments.

Biochemical composition
The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. The best-studied compound is curcumin, which constitutes 3.14% (on average) of powdered turmeric. However, there are big variations in curcumin content in the different lines of the species Curcuma longa (1–3189 mg/100g). In addition, other important volatile oils include turmerone, atlantone, and zingiberene. Some general constituents are sugars, proteins, and resins.[9]

Pharmacology of Turmeric
Turmeric, also known as curcuma longa, is a very common herb. Often referred to as the “Queen of Spices,” its main characteristics are a pepper-like aroma, sharp taste and golden color. People across the globe use this herb in their cooking. According to the Journal of the American Chemical Society, turmeric contains a wide...
range of antioxidant, antiviral, antibacterial, antifungal, anticarcinogenic, antimitogenic and anti-inflammatory properties. It is also loaded with many healthy nutrients such as protein, dietary fiber, niacin, Vitamin C, Vitamin E, Vitamin K, potassium, calcium, copper, iron, magnesium and zinc. Due to all these factors, turmeric is often used to treat a wide variety of health problems.

1. Prevents Cancer: Turmeric can help prevent prostate cancer, stop the growth of existing prostate cancer and even destroy cancer cells. Multiple researchers have found that the active components in turmeric makes it one of the best protectors against radiation-induced tumors. It also has a preventive effect against tumor cells such as T-cell leukemia, colon carcinomas and breast carcinomas.

2. Relieves Arthritis: The anti-inflammatory properties in turmeric are great for treating both osteoarthritis and rheumatoid arthritis. In addition, turmeric’s antioxidant property destroys free radicals in the body that damage body cells. It has been found that those suffering from rheumatoid arthritis who consume turmeric on a regular basis experience much relief from the moderate to mild joint pains as well as joint inflammation.

3. Controls Diabetes: Turmeric can be used in the treatment of diabetes by helping to moderate insulin levels. It also improves glucose control and increases the effect of medications used to treat diabetes. Another significant benefit is turmeric’s effectiveness in helping reduce insulin resistance, which may prevent the onset of Type-2 diabetes. However, when combined with strong medications, turmeric can cause hypoglycemia (low blood sugar). It is best to consult a healthcare professional before taking turmeric capsules.

4. Reduces Cholesterol Level: Research has proven that simply using turmeric as a food seasoning can reduce serum cholesterol levels. It is a known fact that high cholesterol can lead to other serious health problems. Maintaining a proper cholesterol level can prevent many cardiovascular diseases.

5. Immunity Booster: Turmeric contains a substance known as lipopolysaccharide, which helps stimulate the body’s immune system. Its antibacterial, antiviral and antifungal agents also help strengthen the immune system. A strong immune system lessens the chance of suffering from colds, flu and coughs. If you do get a cold, a cough or the flu, you can feel better sooner by mixing one teaspoon of turmeric powder in a glass of warm milk and drinking it once daily.

6. Heals Wound: Turmeric is a natural antiseptic and antibacterial agent and can be used as an effective disinfectant. If you have a cut or burn, you can sprinkle turmeric powder on the affected area to speed up the healing process. Turmeric also helps repair damaged skin and may be used to treat psoriasis and other inflammatory skin conditions.

7. Weight Management: Turmeric powder can be very helpful in maintaining an ideal body weight. A component present in turmeric helps increase the flow of bile, an important component in the breakdown of dietary fat. Those who wish to lose weight or treat obesity and other associated diseases can benefit from having one teaspoon of turmeric powder with every meal.

8. Prevents Alzheimer’s Disease: Brain inflammation is suspected to be one of the leading causes of cognitive disorders such as Alzheimer’s disease. Turmeric supports overall brain health by aiding in the removal of plaque build-up in the brain and improving the flow of oxygen. This can also prevent or slow down the progression of Alzheimer’s disease.

9. Improves Digestion: Many key components in turmeric stimulate the gallbladder to produce bile, which then improves digestion and reduces symptoms of bloating and gas. Also, turmeric is helpful in treating most forms of inflammatory bowel disease including ulcerative colitis. However it is important to bear in mind that people suffering from any kind of gallbladder disease should not take turmeric as a dietary supplement as it may worsen the condition. It is best to consume turmeric in raw form when suffering from a digestive problem.

10. Prevents Liver Disease: Turmeric is a kind of natural liver detoxifier. The liver detoxifies the blood through the production of enzymes and turmeric increases production of these vital enzymes. These vital enzymes break down and reduce toxins in the body. Turmeric also is believed to invigorate and improve blood circulation. All of these factors support good liver health. Given the numerous health benefits of turmeric, adding this powerful herb to your diet is one of the best things you can do to improve the quality of your life. You can add turmeric in powder form to curries, stir fried dishes, smoothies, warm milk and even to spicy salad dressings. Turmeric can be taken in pill form also. However, turmeric should not be used by people with gallstones or bile obstruction.

MATERIALS AND METHOD

Drugs and chemicals: The standard drug 5-FU purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Cell culture: The human prostate cancer cell line DU-145 cell line was provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained
at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Apparatus: Round bottom flask, water condenser, heating mantle, motor and pestle.

Methodology for Soxhlet extraction: First the dried fine turmeric powder is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol, methanol and chloroform is heated on three different water bath or on a heating mental where three Soxlet apparatus set up. As the solvent boil, its vapor rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind.[13] Afterwards the Ethanolic, methanolic and chloroform extracts are transferred in a clean and dried beaker separately and is concentrated by placing on a bath and cool and then ethanolic extract of turmeric powder (EETP), methanolic extract of turmeric powder (METP), and chloroform extract of turmeric powder (CETP) are obtained and keep all these extracts in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Phytochemical screening: Preliminary phytochemical screening of EETP, METP and CETP have shown the presence of diverse bioactive molecules such as: carbohydrates, proteins and aminocids polyphenols, carotenoids, phytosterols and alkaloids which are confirmed by their specific qualitative confirmatory chemical tests.[12-15]

Screening of in vitro cytotoxic activity of EETP, METP and CETP by SRB assay.

Principle[16, 17]
Sulphorodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonic acid group. Under mild acidic conditions SRB dye binds to basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude.

Procedure[18]
The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0x105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and10 µg/ml, 20µg/ml, 30 µg/ml and 40 µg/ml of different concentration of turmeric powder extracts (METP, EETP, CETP) were added to the cell in microtitre plate. The plates were incubated at 37°C for 72 hrs in 5% CO2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hrs, 25µl of 50% TCA was added to wells gently such that it forms a thin layer over the test extracts to form overall concentrations 10%. The plates were incubated at 40c for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with 100 µl SRB and kept for 30 mnts at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10 mM Tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 mnts. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula.

\[
\% \text{cell growth inhibition} = 100 - \{ (\text{At} - \text{Ab}) / (\text{Ac} - \text{Ab}) \} \times 100
\]

At = Absorbance value of test compound.
Ab = Absorbance value of blank.
Ac = Absorbance value of control.

RESULTS AND DISCUSSION
The results for cell growth inhibition by various extracts of turmeric powder such as METP, EETP and CETP against DU-145 cell lines for various concentrations is shown in table 1, 2 and 3. As the concentration increases there is an increase in the cell growth inhibition and it was found that METP, with the highest 92.80% growth inhibition at 10 µg (IC50 = 2.5 µg/ml), EETP with the 93.83% growth inhibition at 10 µg (IC50 = 2.3 µg/ml) and CETP with the 94.52% growth inhibition at 10 µg (IC50 = 2.1 µg/ml). The IC50 value of standard drug 5-FU was found to be 1.5 µg/ml with 96.54 % growth inhibition at concentration 50 µg/ml.

IC50 determination[68]: IC50 is the acronym for “half maximal inhibitory concentration”. IC50 value indicates the concentration needed to inhibit a biological or biochemical function by half (e.g. inhibition of enzymes, affinity to cell receptors). Amongst others, determination of IC50 is commonly calculated via linear interpolation: The activity of an enzyme is determined after exposure to a series of inhibitor concentrations. IC50 is calculated by the following formula:

\[
\text{IC50} = (50\% \cdot \text{Low Inh\%}) / (\text{High Inh\%} - \text{Low Inh\%}) \times (\text{High Conc-Low Conc}) + \text{Low Conc}
\]

Low Inh% / High Inh% : % inhibition directly below / above 50% inhibition
Low Conc / High Conc: Corresponding concentrations of test compound.
Table 1: For percentage (%) of cell Growth Inhibition of Methanolic Extract of Turmeric (Curcuma longa) powder (METP) on DU-145 Cell lines by SRB Assay.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Concentration of the Extracts</th>
<th>Absorbance of extracts</th>
<th>Inhibition of cell growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 µg/ml</td>
<td>0.021</td>
<td>92.80</td>
</tr>
<tr>
<td>2</td>
<td>20 µg/ml</td>
<td>0.029</td>
<td>90.06</td>
</tr>
<tr>
<td>3</td>
<td>30 µg/ml</td>
<td>0.032</td>
<td>89.05</td>
</tr>
<tr>
<td>4</td>
<td>40 µg/ml</td>
<td>0.036</td>
<td>87.67</td>
</tr>
<tr>
<td>5</td>
<td>50 µg/ml (5-FU)</td>
<td>0.0101</td>
<td>96.54</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>0.292</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 6: Percentage (%) of cell growth inhibition by METP on human prostate cancer DU-145 cell line.

Table 2: For percentage (%) of cell Growth Inhibition of Ethanolic Extract of Turmeric (Curcuma longa) powder (EETP) on DU-145 Cell lines by SRB Assay.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Concentration of the Extracts</th>
<th>Absorbance of extracts</th>
<th>Inhibition of cell growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 µg/ml</td>
<td>0.018</td>
<td>93.83</td>
</tr>
<tr>
<td>2</td>
<td>20 µg/ml</td>
<td>0.027</td>
<td>90.75</td>
</tr>
<tr>
<td>3</td>
<td>30 µg/ml</td>
<td>0.030</td>
<td>89.72</td>
</tr>
<tr>
<td>4</td>
<td>40 µg/ml</td>
<td>0.035</td>
<td>88.01</td>
</tr>
<tr>
<td>5</td>
<td>50 µg/ml (5-FU)</td>
<td>0.0101</td>
<td>96.54</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>0.292</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 7: Percentage (%) of cell growth inhibition by EETP on human prostate cancer DU-145 cell line.
Table 3: For percentage (%) of cell Growth Inhibition of Chloroform Extract of Turmeric (Curcuma longa) powder (CETP) on DU-145 Cell lines by SRB Assay.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Concentration of the Extracts</th>
<th>Absorbance of extracts</th>
<th>Inhibition of cell growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 µg/ml</td>
<td>0.016</td>
<td>94.52</td>
</tr>
<tr>
<td>2</td>
<td>20 µg/ml</td>
<td>0.025</td>
<td>91.43</td>
</tr>
<tr>
<td>3</td>
<td>30 µg/ml</td>
<td>0.028</td>
<td>90.41</td>
</tr>
<tr>
<td>4</td>
<td>40 µg/ml</td>
<td>0.032</td>
<td>89.04</td>
</tr>
<tr>
<td>5</td>
<td>50 µg/ml (5-FU)</td>
<td>0.0101</td>
<td>96.54</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>0.292</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 8: Percentage (%) of cell growth inhibition by CETP on human prostate cancer DU-145 cell line.

Fig 9: Graphical representation of Percentage (%) of cell Growth Inhibition by METP, EETP and CETP on DU-145 Cell line at different concentration.

CONCLUSION

The results obtained from the present studies displayed that the Preliminary Phytochemical screening of various extracts of turmeric powder such as METP, EETP and CETP had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols and alkaloids etc and the results obtained from the in-vitro studies performed by SRB assay against human prostate cancer cell line DU-145 displayed that the various extracts of turmeric powder such as METP, EETP and CETP possessed a very good cytotoxic activity. From the present studied it had been concluded that METP, EETP and CETP, all were exhibiting the potential capability to inhibit the cancer cell when compared with standard drug 5-FU and the cell growth inhibition of METP, EETP and CETP were found to be the highest 92.80% at 10 µg (IC_{50} = 2.5 µg/ml), 93.83% at 10 µg (IC_{50} = 2.3 µg/ml) and 94.52% at 10 µg (IC_{50} = 2.1 µg/ml).

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