Identification of signals mediated the treatment of cancer by curcumin extract.

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Abstract

Background: The plant polyphenols play a vital role in human diets that link to health and resilience evolutionarily and mechanistically.

Materials and methods: The rational of this study to identify the signals mediated the treatment of cancer by curcumin extract.

Results: Different cancer cell line will be used for detection of the signal molecules of apoptotic during treatment with Methanol Extract of Curcumin (MEC). Hep2G and HeLa cell lines were used treated with 10, 20 and 40 μl of MEC. Analysis of MEC by GC/MS referred presence of 1, 3, 5-benzenetriol as active ingredients. In vitro study, the anticancer activity of 1, 3, 5-benzenetriol were 87% and 85% for HeLa and HepG2 respectively.

Conclusion: Curcumin as an efficient medicinal displayed a significant effect on HeLa and HepG2 cells which is very effective in the treatment of cancer this is due to presence of 1, 3, 5-benzenetriol.

Keywords: Curcumin extract, Anticancer, Signal transduction.

Introduction

Plant polyphenols are a structurally and functionally diverse family of secondary natural products shaped by adaptive evolution as chemical responses to biotic and abiotic factors impinging on the sessile life style of plants [1]. The role of plant natural products in plant survival, the co-evolution of animals and plants the role of animals in plant reproduction and dispersal, and the presence of plant polyphenols in human diets link plant and human health and resilience evolutionarily and mechanistically [2]. Polyphenol biosynthesis provides a plethora of compounds that collectively reduce chronic inflammation in humans. Polyphenols in plant fruits comprise flavanones, flavones, flavanols, flavan-3, 4-diols, catechins, proanthocyanidins, anthocyanidins and stilbenes [3]. Additionally, these core chemical structures are extensively amended by biosynthetic tailoring reactions encompassing regioselective oxidative couplings, methylations, acylation, glycosylation and prenylation [4]. These latter alterations often impart distinct bioactivity-bioavailability properties on the core polyphenolic scaffolds and contribute to synergistic multi-component interactions in more complex mixtures [5].

Curcumin extracted from the rhizomes of a perennial herb *Curcuma longa* with wide leaves has gained immense interest in the scientific community due to its anti-bacterial anti-fungal anti-viral, anti-cancer, anti-inflammatory, anti-malarial, anti-oxidant and wound healing properties. Asian countries like India and China have used turmeric as a traditional medicine for nearly 2000 years in the form of a paste or an oral form to treat various ailments of skin and illnesses successfully until the modern medicine found it prominent in the last two centuries curcumin was isolated in crystalline form [6].

Signal transduction is essential for all living cell function [7] development [8], differentiation [9], apoptosis and cell death. Signaling molecules including hormones, neurotransmitters, and growth factors [10]. Signals mediated by a growth factor involve binding to its receptor initiates a process that starts with the binding with membrane or intracellular receptor [11]. The amplified signal is then propagated to the nucleus, resulting in induction or repression of gene expression [12]. Mitochondrial dysfunction was suggested to be related with many chronic diseases. The rational of this study to identify the signals mediated the treatment of cancer by curcumin extract. Oxidative damage is often related with path physiology of many diseases [13].

Understanding the active anticancer ingredients of curcumin is important biological processes as gene expression, protein synthesis, development, carcinogenesis and apoptosis.

Materials and Methods

**Identification of MEC by GC/MS**

Methanol Optima LC/MS grade from Fisher (NJ, USA) and hexane 95% for organic residue analysis from Avantor (PA,
USA) were used for sample preparation GC/MS 5975 (Agilent, CA, USA) system including Agilent 7890 A gas chromatography.

Assessment of antitumor activity of cell culture and treatment

HeLa and HepG2 and non-cancerous BHK (Baby hamster kidney) cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cell lines were maintained in a humidified incubator with 5% CO₂ at 37°C and grown in Dulbecco’s Modified Eagle’s Medium (DMEM) (UFC biotech, Riyadh, KSA) for MCF-7 and BHK and in RPMI 1640 (UFC biotech, Riyadh, KSA) for Jurkat cells. All were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) less than 0.1% in both untreated and treated cells. Antibiotics (100 units/ml) (Gibco U.S.A). A 100 mM solution of studied compounds was prepared in 100% DMSO (Dimethyl sulfoxide, Gibco U.S.A) or water. From the stock solution, appropriate working concentrations were prepared in the culture media where the final concentration of DMSO was less than 0.1% in both untreated and treated cells.

Cell viability assay

The cell proliferation assay test is a colorimetric assay that is based on the break of a tetrazolium salt (WST-1) by dehydrogenase in mitochondria to form formazan in viable cells. The higher the number of viable cells, the higher the percentage of cell viability was calculated by assuming control untreated) samples as 100 % viable [14]. The IC₅₀ (50% inhibitory concentration) values were calculated from the dose-response curve.

Apoptosis assay

HeLa and HepG2 cells were plated in a 96-well plate at a density of 4 × 10⁴ cells/well and incubated for 24 h. After treating the cells with desired concentration of the drugs for several time periods, Annexin V/7AAD (Millipore®, Billerica, MA) staining was carried out as per the manufacturer’s protocol. Briefly, 100 µL of nixin reagent (Millipore®, Billerica, MA) staining solution (containing annexin V fluorescein and 7AAD) was added and incubated at room temperature for 20 min in dark surroundings. The forward and side scatter was recorded at 10,000 events and the subsequent percentage of the early and the late apoptotic cells was analysed using the Guava® easyCyte 12 HT Benchtop Flow Cytometer (Millipore®, Billerica, MA) and the results plotted using the InCyte® software (Millipore®, Billerica, MA).

Statistical analysis

Results were expressed as mean ± SE. Triplicates teats were done in the same experiment. Student’s t-test was calculated for comparison between the pairs. One way Analysis of Variance (ANOVA) was performed using SPSS version 15.

Results and Discussion

GC/MS analysis of MEC

Figure 1 GC/MS analysis of MEC showed that, measure RI at 1373 and reference RI at 1342. This signal is specific for 1, 3, 5-benzenetriol that referred to C₆H₄O₃.

Anti-proliferation activity of MEC

Curcumin can exert its effect by initiating either intrinsic (mitochondrial) or extrinsic (receptor mediated) apoptotic pathways. The tumor suppressor p53 plays a pivotal role in initiating a caspase cascade through the immediate activation of pro-apoptotic proteins of B-cell lymphoma 2 (Bcl-2) family like Bcl-2 associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak). These Bax and Bak perforate the mitochondrial membrane and hence release the cytochrome-c into the cytoplasm promoting mitochondria-mediated apoptosis. Curcumin also has the ability to inhibit anti-apoptotic proteins like Bcl-2 and B-cell lymphoma extra-large (Bcl-xL) [15].

Molecular docking studies conducted by Luthra et al. confirmed the inhibition of Bcl-2 by direct binding of curcumin to the second cavity of the protein through the intermolecular interactions of amino acids [16]. It was demonstrated that the curcumin inhibited the growth of colorectal carcinoma LoVo cells by initiating a caspase cascade at a considerable concentration of 0-30 µM. Moreover the nuclear staining by Annexin V/PI was found positive.
confirming the activation of apoptotic machinery. Recently, several reports have confirmed the anti-proliferative action of curcumin in cancers of breast, pancreas and lung through the down-regulation of Epidermal Growth Factor Receptor (EGFR) which is usually highly up-regulated in tumor environments [17].

The cell cycle is tightly regulated by a group of cell cycle regulatory proteins like cyclins and cyclin-dependant kinases (Cdk) which aid in the cell proliferation of the cells. But in tumors these regulatory proteins are up-regulated hence, leading to the rapid proliferation of cells. However, curcumin exerts its inhibitory effect on these regulatory proteins and inhibits the cell proliferation eventually [18].

Accumulating evidence shows that curcumin is not only anti-proliferative or inhibits the reactive oxygen species but also found to promote apoptosis via induction of expression of different second messengers like nitric oxide synthase, COX-2, Matrix Metalloproteinase-2 (MMP-2) and Matrix Metalloproteinase-9 (MMP-9) in cancer cells. Sethi et al. have reported that abnormal inflammatory signaling pathways also play a pivotal role in the advancement and growth of several cancers [19].

The main objective of the research group is to serve the community by developing smart foods and diets that enhance quality of life and human health. In addition, build strong ties with organizations and institutions interested in these aspects. Main measures of the proposal will contribute to the overall objective by: capacity building, networking and twinning among research groups, research centres and institutions and linking to economic and social environment.

To determine the selectivity of MEC, the normal BHK cells were exposed to the similar concentrations of 1, 3, 5-benzenetriol that was used with cancer cells for 24 h. At a concentration of 10, 20 and 40 µM, significantly caused cell proliferation inhibition of HeLa cells by about 91%. However, increasing the concentration to 20 µM induced a cell proliferation inhibition in HeLa and Hep2G by about 85% and 87% respectively. The cell viability at the highest concentration of 40 µM was found to be 23.3%, 33%, respectively for a time incubation of 24 h respectively.

Previous study found that inhibitory effect of on HepG2 and HeLa can be enhanced with the addition of curcumin. The mechanism curcumin may reduce the oxidation reaction. GC/MS analysis of MEC showed that, measure RI at 1373 and reference RI at 1342. This signal is specific for 1, 3, 5-benzenetriol that referred to C₆H₆O₃.

Secondly, curcumin, itself has obvious effect of inhibiting tumor. Rezq et al. demonstrated that curcumin can make tumor cells sensitive, especially human colon carcinoma cells [12]. When different concentration of 1, 3, 5-benzenetriol were used, the inhibitory rate was decreased by 20.587%, 22.6% respectively. The synergistic anticancer effects fall decreased slowly with decreasing concentration of 1, 3, 5-benzenetriol. However, the inhibition rate of the mixture wills no longer increase that may be associated with low substrate concentration [20]. Hence, for the longer antitumor activity of the capsule it is advised to increase the concentration of 1, 3, 5-benzenetriol in the treatment therapy (Table 1 and Figure 2).

### Table 1. IC₅₀ values (µM) of the microcapsules against tumor cells and the normal cells are presented as mean ± SE. Experiment was done triplicate *p<0.05.

<table>
<thead>
<tr>
<th>Conc of MEC (µM)</th>
<th>HeLa</th>
<th>Hep2G</th>
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<tbody>
<tr>
<td>10</td>
<td>30.30 ± 2.1a</td>
<td>48.31 ± 3.4a</td>
</tr>
<tr>
<td>20</td>
<td>17.15 ± 0.5a</td>
<td>53.38 ± 2.1a</td>
</tr>
<tr>
<td>40</td>
<td>19.20 ± 1.7a</td>
<td>27.27 ± 1.5a</td>
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Thymoquinone (40 µM) 14.75 ± 5.3 12.51 ± 1.7

**Figure 2. Antiproliferation activity of different concentrations of 1, 3, 5-benzenetriol.**

### Detection of apoptosis

Annexin V-7AAD staining of the HeLa cells which were treated with increasing concentrations 10, 20, 14 µM for 24 h was detected. Increasing the concentration1, 3, 5-benzenetriol was associated with an increase in the number of apoptotic cells. It was found that, began to significantly trigger apoptosis at 10 µM. While at a concentration of 40 µM, the percentage of the annexin V positive cells was higher and approximately consistent with the value detected in cell viability assays. The present study involved the 1, 3, 5-benzenetriol of curcumin. The compounds showed potent effect on HeLa and Hep2 G cells with significantly higher anti-cancer activity. Taken together, curcumin-1, 3, 5-benzenetriol as promising in treatment of some cancer types.

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### References


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