Management of hepatocellular carcinoma development by *Trianthema portulacastrum* extract; *in-vitro* evidence

Walid Said¹, Abeer A. Khattab¹, Saadia H. Aly¹, Hany Khalil² and Sabah A. Abo-Elmaaty¹*  
¹Botany and Microbiology Department, Faculty of Science, Benha University, Egypt.  
²Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute,  
Sadat University, Egypt.  
E-mail: waleed.essa17@fsc.bu.edu.eg

Abstract

Liver cancer remains a worldwide concern, with more than 1 million new cases expected by 2025. Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver cancer, making up around 80% to 90% of all primary liver cancers, whereas the other categories include cholangiocarcinoma (10-20%) and angiosarcoma (1%). Herbal treasure contains a number of novel phytochemicals that can be employed both therapeutically and preventively to address a variety of liver-related abnormalities, including HCC. *Aim:* To look into the prospective activity of *Trianthema portulacastrum* as a folkloric plant against HCC. *Methods and materials:* The extract of *T. portulacastrum* was prepared using dimethyl sulfoxide (DMSO). The cytotoxic activity of *T. portulacastrum* DMSO extract on HepG2 cell lines was affirmed using the MTT assay when compared to normal cells. It was trialled *in-vitro* for HCC activity against MEK-1 using quantitative real-time polymerase chain reaction (qRT-PCR). *Results:* It showed promising inhibitory activity against MEK-1 gene expression without any detectable cytotoxic effects. It significantly blocked the Ras/Raf/MEK/ERK tumor proliferation signaling pathway and induced an apoptotic signaling pathway. *Conclusion:* The *T. portulacastrum* DMSO extract inhibited the HepG2 cells proliferation and showed anticancer activity against HCC.

**Keywords:** Hepatocellular carcinoma, cytotoxic activity, Ras/Raf/MEK/ERK signaling pathway, *Trianthema portulacastrum*.

1. Introduction

Hepatocellular carcinoma (HCC), the most common type of malignant liver tumor, is one of the deadliest human cancers and the leading cause of cancer death worldwide. There has been an uptick in HCC incidence and death rates that are nearly equal to the preceding four decades in the world [1]. This horrible disease has a truly dreadful outcome, and many HCC patients' treatments were postponed due to the muddled nature of early symptoms. The primary treatments for liver cancer are currently surgery and chemotherapy, which have the aptitude to increase the 5-year rate of survival to 75%, (compared to 30% before these therapies), but still, the curative effects of the available chemotherapeutic agents are insufficient, and they have significant adverse effects. Anticancer therapy is one of the most challenging tasks in healthcare, and therefore the quest for remarkably effective antitumor medicines continues to be a hot topic of inquiry. Researchers have focused their attention on naturally occurring chemicals since they have been regarded as having quite a few undesirable side effects compared to traditional therapies such as chemotherapy [2].

Herbal plants are wellsprings of bioactive principles, notably flavonoids, which have far-reaching been implicated with anti-cancer activity [3]. The global demand for herbal medicine is not only high but also growing. The active development of innovative therapeutic techniques and molecularly targeted medicines using herbal medicine may provide a chance to search the agents in HCC, providing new prospect for the future [4]. *Trianthema portulacastrum* L., a member of the Aizoaceae, is a common weed with extensive traditional usage against diseases due to having various bioactive principles, notably flavonoids that already have natural anti-microbial, anti-inflammatory, antioxidant, anti-cancer, and other bioactivities It was outlined as a perennial or annual fleshy herb that is spreading in agricultural fields [5] [6].

Carcinogenic process biomarkers are characterized by abnormal protein signaling pathways that make a significant contribution to unregulated cell proliferation, differentiation, survival, and apoptosis. The Ras/Raf/MEK/ERK cascade reaction is a crucial MAPK signaling mechanism for signal pathway conclusion that is affiliated with in cell cycle regulation, apoptosis, and distinctions. Its precise functional role in the context of this intricate signaling network and HCC carcinogenesis is still unknown. Multiple stimuli can activate the corresponding cell surface receptors, culminating in the signal transduction pathway and resulting in an appropriate biological response [2]. The Ras/Raf/MEK/ERK signaling pathway has already yielded additional insight and potential therapies against HCC. Autophagy is a cellular building maintenance action which employs lysosomal breakdown in order to keep cells alive and energy sources available. Autophagy has indeed
been implicated in the onset and progression of several human diseases in recent decades. Not only does autophagy play a central role in the physiology of normal liver (as unfolded proteins protein authorization and energy and nutrient metabolism in liver cells), but it has a role in the evolution of liver diseases such as non-alcoholic and alcoholic fatty liver, drug-induced liver injury, protein posttranslational liver diseases, viral hepatitis, fibrosis, ageing, liver cancer, and liver ischemia-reperfusion injury too [7]. Apoptosis, furthermore known as programmed cell death (PCD), describes the changes that occur in cells in response to intrinsic or extrinsic stimuli in order to maintain the molecular balance required for disease control [8]. Autophagy and apoptosis are both genetically modulated biological systems which govern cell proliferation and sustenance and are required for cell fate maintenance. Manipulation of the autophagy mechanism may be used to control malignant cell behavior and induce apoptosis [9] [10] [11].

2. Aim of the work
To evaluate the cytotoxic and anticancer activity of T. portulacastrum DMSO extract against normal hepatocytes and HepG2 cells.

3. Materials and Methods

Plant material and extraction
Fresh leaves of T. portulacastrum were obtained from some fields of EL-Qanatir in Qalubiya during summer 2020. After sterilizing the samples with 75% ethanol and grinding them with liquid nitrogen, they were dissolved in 1 mL of DMSO to achieve a final concentration of 100 mg/mL. The final extraction 0 was stored at 4°C for prospective use.

Cell lines
The HepG2 cell line were obtained from the Egyptian holding company for biological products and vaccines, VACSERA, Dokki, Egypt, and propagated in RPMI media containing 4 mM L-glutamine, 4 mM sodium pyruvate, and 5% heat-treated bovine serum albumin (BSA). Normal liver cells were propagated in RPMI media which contains 4 mM L-glutamine and 10% BSA. These cell lines were incubated at 37°C with 5% CO₂ [12] [13].

Cell proliferation progress
1st day: HepG2 was maintained and prepared. After removing the old medium RPMI media, HepG2 cells were washed with 10 mL of PBS, then supplemented with 2 mL of trypsin, and incubated at 37°C for 2 minutes in a 5% CO₂ incubator. Then 6 mL of RPMI media was added to the HepG2 cell lines.

2nd day: The incubated HepG2 were evaluated for cell proliferation and were also examined for any morphological changes under an inverted microscope.

Stock preparation
HepG2 cells were transferred and then rinsed with PBS. The cells were incubated for 2 minutes after adding trypsin. The prepared medium was then added, mixed, and divided into four Eppendorf tubes in 6 mL increments. The remaining cells were then centrifuged at room temperature for five minutes at 5000 RPM and then 500 μL of BSA and 10 μL of DMSO were added to all tubes. Finally, before using the stock again, it was incubated in liquid nitrogen.

Extract treatment and starvation procedure
1st day: HepG2 cell lines were washed before being seeded in a 6-well plate with trypsin. The cells were divided into 100.0000 cells per well in 2 mL of RPMI medium and incubated overnight in a CO₂ incubator.

2nd day: All cells were propagated with different concentrations of the extractions (0-4 mg/mL). HepG2 cells were starved by incubating them in PBS instead of RPMI medium for 1 hour, 2 hours, or 24 hours. For 24 hours, HepG2-treated cells and starved cells were incubated in a CO₂ incubator.

Quantitative real time polymerase chain reaction technique
By using qRT-PCR the genes expression was quantified, and the cellular total RNA was extracted with Triazole (Invitrogen, USA) and purified with an RNA purification kit (Invitrogen, USA). From 1 g of total RNA, complementary DNA (cDNA) was generated using an M-MLV reverse transcriptase (Promega, USA). MEK-1 messenger RNA (mRNA) expression was measured using the Quanti-Test-SYBR-Green PCR Kit (Qiagen, USA). The primers are given in table 1. Signaling cascades of Ras/Raf/MEK/ERK are triggered by growth factors from their receptors to specific transcription factors that induce apoptotic, and control gene expression signaling pathways. MEK-1 activity could biochemically inhibit, regulate cell proliferation and induce apoptosis. By using the housekeeping glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression level for standardization in the real-time PCR data analysis. The reaction of PCR which includes 10 μL SYBR green, 0.25 μL of RNase inhibitor (25 U/μL), 0.2 M of each primer, 2 μL of synthesised cDNA, and nuclease-free water to a final volume of 25 μL. PCR condition (95°C for 10 minutes), followed by 40 cycles of (95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds) [8] [14].

Table (1) Primer sequences used for mRNA quantification of indicated genes

4. Data analysis

The final chart was created using Microsoft Excel. The following equations (Delta-Delta Ct analysis was employed in the quantification analysis of mRNA obtained from qRT-PCR technique (1) delta - Ct = Ct value for gene - Ct value for GAPDH, (2) delta - delta Ct = delta Ct value for experiment - delta Ct for control, (3) Quantification fold change = (2 - delta-delta Ct) [7] [15]. P-values ≤ 0.05 were deemed statistically significant. The relevance of all RT-PCR data was determined statistically using the student's two-tailed t-test. SDS2.2.2 software was employed to produce Ct values for possible gene expression using delta-delta Ct equations from the qRT-PCR data.

5. Results

The level of MEK-1 gene expression in HepG2 cell lines following treatment with DMSO and T. portulacastrum extract as depicted in Table 2. MEK-1 relative gene expression was detected in overnight-treated cells using qRT-PCR. The fold change in treated cells over DMSO-treated and non-treated cells demonstrated that the extract significantly reduced the relative expression of the MEK-1 gene (Figure 1). T. portulacastrum extract successfully suppressed the Raf/MEK/ERK signaling pathway in liver cancer cells, indicating a possible synergistic action.

Table 2 The expression level of MEK-1 gene of HepG2 cell line.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Treatment</th>
<th>Expression fold changes</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>MEK-1</td>
<td>DMSO</td>
<td>1.15</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Tp</td>
<td>0.37</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

MEK: Mitogen-activated protein kinase, NT: non-treated, DMSO: dimethylsulphoxide, and Tp: Trianthema portulacastrum. *: significant at P-values ≤ 0.05

Fig. (1) The relative gene expression of MEK1 in treated HepG2 cells in comparison with untreated cells.

Error bars indicate SD of three independent experiments.

MEK: Mitogen-activated protein kinase NT: non-treated, DMSO: dimethylsulphoxide, and Tp: Trianthema portulacastrum. *: significant at P values ≤ 0.05.

6. Discussion

Multiple published studies have proved the effectiveness of several herbal remedies for a wide range of diseases. There are numerous naturally produced herbal medicines available for cancer patients. Since the most of chemotherapeutic drugs were toxic to normal cells, drug resistance developed. As a result, scientific research and testing of traditionally used cancer treatment herbs could provide a significant source of new chemotherapeutic drugs [16]. T. portulacastrum extract was encountered to have anticancer properties in HepG2 cells by regulating apoptosis. Several studies have shown that several extracted compounds from T. portulacastrum leaf extract are effective, and the plant may be used as an anticancer, antioxidant, and antibacterial agent. The current study demonstrated anticancer activity of T. portulacastrum DMSO leaf extract by successfully suppressing the proliferation signaling pathway (Ras/Raf/MEK/ERK) and regulating HepG2 cell growth by trying to restore the prolonged Ras/Raf/MEK/ERK signaling pathway and controlling PCD. The green leaves and flowers of guava (Psidium guajava) are disrupted by DMSO extract. After infection, a virus replicates by activating P53 and its apoptotic-related factors [8]. Hymenosporum flavum leaf extract inhibited the expression of RAF-1 and ERK-2 genes [3]. T. portulacastrum extract significantly blocked MEK-1 gene expression.

7. Conclusion

Our study confirmed the T. portulacastrum extract’s in-vitro activity against HCC. This study’s outcomes will be beneficial in future research, and T. portulacastrum DMSO leaf extract may be
promising for further preclinical and clinical studies for HCC treatment.

Conflict of interest statement
The authors declare that they have no conflict of interest.

Ethical approval
An informed consent was obtained from all participants before being enrolled in the study.

Authors contribution
All authors are equally contributed.

References