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Original Research Article

Anticancer Potentiality of *Ferula assa-foetida* Gum Extract against CT-26, HT-29, SW742 and WiDr Colorectal Cancer Cell Lines

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Abstract: ***Background*:** *Ferula assa-foetida* L has several therapeutic effects such as anticancer traits. The aim of this study was investigation of anticancer effect of *Ferula assa-foetida* gum alcoholic extract against CT-26, HT-29, SW742 and WiDr colorectal cancer cell lines. ***Methods*:** The hot extract was obtained by soxhlet using 70% ethanol solvent. Various cell lines were cultured in DMEM medium and in vitro anticancer effects using MTT assay was performed. A dilution (5, 10, 25, 50 and 100µg/mL) of the gum extract were subjected to cell lines in triplicate. and incubated for 24, 48 and 72hrs. The main phytochemicals within the gum extract were detected through Gas and mass chromatography. ***Results*:** cytotoxicity rates were observed at 50µg/mL and >100µg/mL, respectively for all cell lines. And morphological change was noticed after 72 hr. application of the gum extract on SW742 cell line. The compounds found in the *Ferula assa-foetida* L mostly included oxygen-containing sesquiterpenes (45.25%) and sesquiterpenes (30.45%). ***Conclusion*:** the viability decreasing was observed for all examined colorectal adenocarcinoma cell lines including SW742, CT-26, HT-29 and WiDr lines depend on time and concentration of the applied extract of *F. assa-foetida* gum. The potent cytotoxicity was observed in 50-100 µg/Ml. Furthermore, a morphological change was detected after 72 hr. on SW742. A high percentages of anticancer compounds which were detected within the gum extract supports the idea of purifying these components and considering them in cancer drugs' phytochemistry.

Keywords: Anticancer traits, apoptosis, colorectal cancer, gum extract, phytochemicals.

INTRODUCTION

Colorectal cancer (CRC) is among those leading causes of cancer around the world which has enhancing trend in recent decades [1, 2]. The CRC occurs more commonly in developed and industrialized countries despite progress in cancer prevention, accurate diagnosis and chemotherapy. Various chemical agents which produce free radicals such as hydroxyl (OH), anion superoxide (O) and hydrogen peroxide (H2O2) compounds lead to mutations in the genetic background of cells and evoke them to become cancerous [3]. Hence, anti-oxidant compounds counteract to free radicals and exert anticancer effects. Nevertheless, chemotherapy is used for treatment, resistance development to these drugs and associated side effects has preferred to application of alternative compounds such as herbal medicines (HMs) [4]. Since the toxicity and side effects of many plant derived antioxidants are less than in synthetic drugs, they can be consumed as a diet and powerful cancer preventive and treatment agent [5]. Considering this, recent efforts to identify and apply novel bioactive herbal compounds with anti-oxidant and anti-cancer properties have been fulfilled.

Ferula assa-foetida (the genus *Ferula* and the Umbeliferae family) is an important perennial pharmaceutical herb which is innate to Iran and central Asia, it includes about 170 species globally, it is an herbaceous and monoecious plant that provides an unpleasant perfume. This genus grows up to 1.5 -2 m, and found in two taste forms, sweet and bitter [6].

One of the plant bioactive compounds is oleo gum- resin (OGR), it exudes from stems and roots after incision them. It has sulfur-like odor and a bitter taste [7]. The asafoetida is consumed by patients in several countries as an anticancer agent. Notably, some fractions such as phenolic compounds (as secondary metabolites) are with high potential of scavenging free radicals [7, 8].

The anti-cancer and cytotoxic effects of *Ferula assa-foetida* L have been studied in various cancers [7-9]. Major antioxidant compounds in the *Ferula assa-foetida* L include phenolic compounds such as carotenoids, flavonoids tocopherols, phenolic acids (benzoic acid and cinnamic acid derivatives) and diterpenes [10]. This study aimed at assessment of anticancer effects of various extracts of *Ferula assa-foetida* L against SW742, CT-26, HT-29 and WiDr CRC lines and to specify the concentration which gave the significant cancer cytotoxicity.

METHODS

Extraction and compounds identification

The *F. assa-foetida* gum was purchased from an Iranian center, Ilam city. The extraction of bioactive compounds of alcoholic extract of *Ferula assa-foetida* was performed using 100mL of 70% ethanol using Soxhlet, extraction procedure was carried out for 4 hr., to obtain a gummy residue, the extract was filtered and the solvent was evaporated on a rotary evaporator under elevated pressure. Afterwards the resulted extract was placed in a vacuum desiccator to get rid of the residual of solvent and moisture. The resulted product was stored in refrigerator prior to analysis [11].

Figure 1: The whole shape of *Ferula assa-foetida* tree https://www.planetayurveda.com/library/hingu-ferula-asafoetida/

Anticancer effects against CRC cell lines

This test was carried out according to [12].Various cell lines were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and penicillin and streptomycin antibiotics and incubated at 37°C with 5% CO2 for 24h.in order to evaluate anticancer efficacy of the gum extract, MTT assay was performed. Value of 10^4 CFU/mL of CRC cells were cultured into DMEM medium in 96-well plates and various concentrations (5, 10, 25, 50 and 100µg/mL) of the gum extract were subjected to wells in triplicate and incubated for 24, 48 and 72hrs. The wells were emptied and 200 µL of DMEM medium and 20 µL of 5mg/mL MTT dye were added and incubated for 4hr. the supernatant was removed and 100µL of DMSO was added and absorbance rate was measured using multi–mode reader Synergy HTX device at 540nm. The viability of cells was measured using the following formula: Viability (%): OD samples [treated cell lines] /OD controls [untreated cell lines]×100

Furthermore, Cell cytotoxicity was observed using light microscopy. Gas chromatography and mass spectrometry (GC /MS) test.

In order to identify the main phytochemicals within the gum of *Ferula assa-foetida*, the extract was subjected to gas chromatography and mass spectrometry. Depend on their mass, the detected chemical compounds have been established compared with NIST library search and registered standards the total procedure detailed in [13].

RESULTS

Cell Cytotoxicity

The 50% and 90% cytotoxicity rates were observed at 50µg/mL and >100µg/mL, respectively for all experimented cell lines. The MTT test results and cellular effects have been represented in Figures 2-5 furthermore, rounding of cells was observed after 72 hr. application of the gum extract on SW742 as displayed in Figure 6.

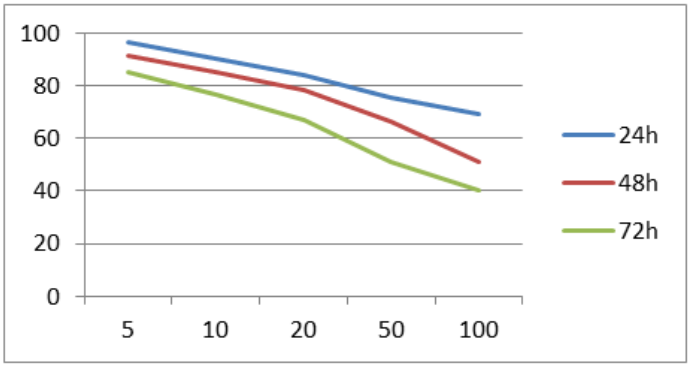

Figure 2: The viability of SW-742 cells treated with different concentrations of *Ferula assa-foetida* L gum alcoholic extract for 24, 48 and 72hrs

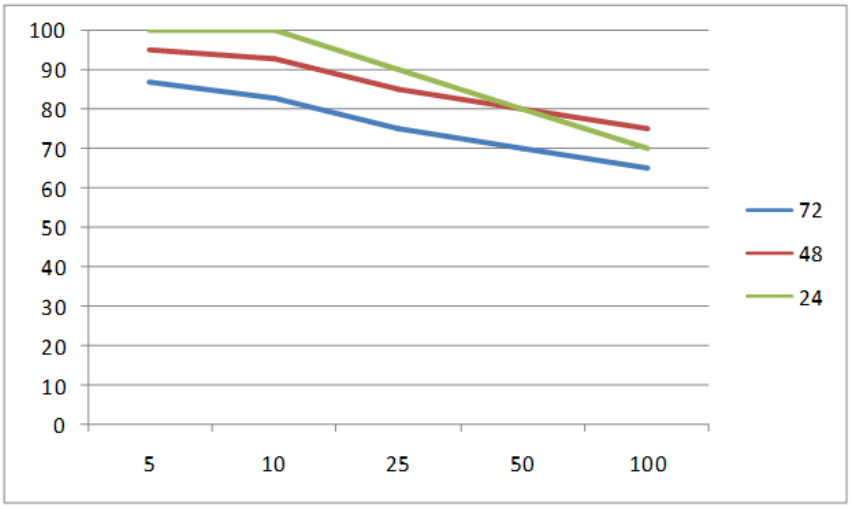

Figure 3: The MTT test of *Ferula assa-foetida* L alcoholic extract at 24, 48 and 72hrs against the CT-29 cell line

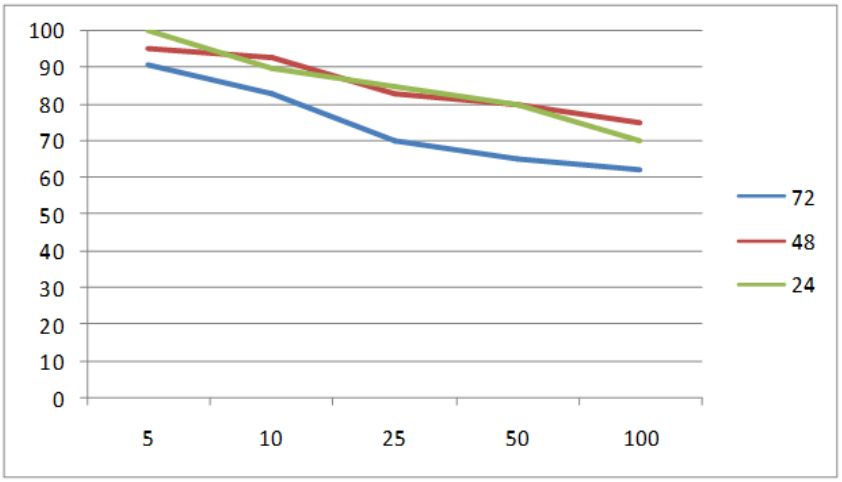

Figure 4: The viability of HT-29 cells treated with different concentrations of *Ferula assa-foetida* L gum alcoholic extract for 24, 48 and 72hrs

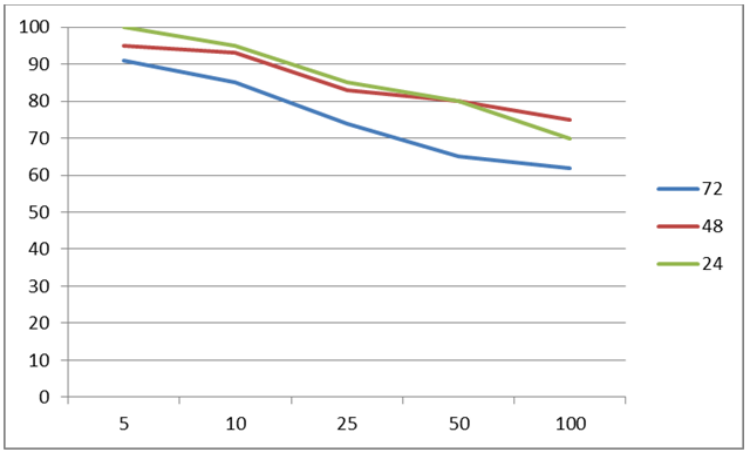

Figure 5: The viability of WiDr cells treated with different concentrations of Ferula assa-foetida L gum alcoholic extract for 24, 48 and 72hrs

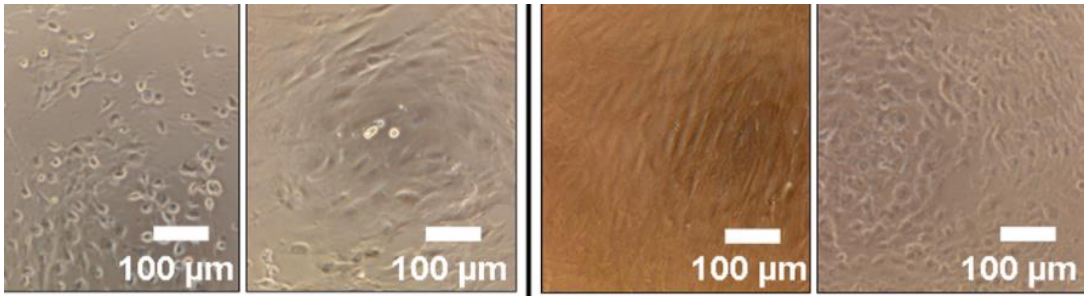

Figure 6: Morphological features of SW742 cell culture affected by the extract of *F. assa-foetida* gum, the left photo after 72 hr

Gas and mass chromatography

The compounds found in the gum of *Ferula assa-foetida* mostly included oxygen-containing sesquiterpenes (45.25%), sesquiterpenes (30.45%), oxygen-containing monoterpenes (13.41%), monoterpenes (1.67%) and others (9.16%) (Figure 7).

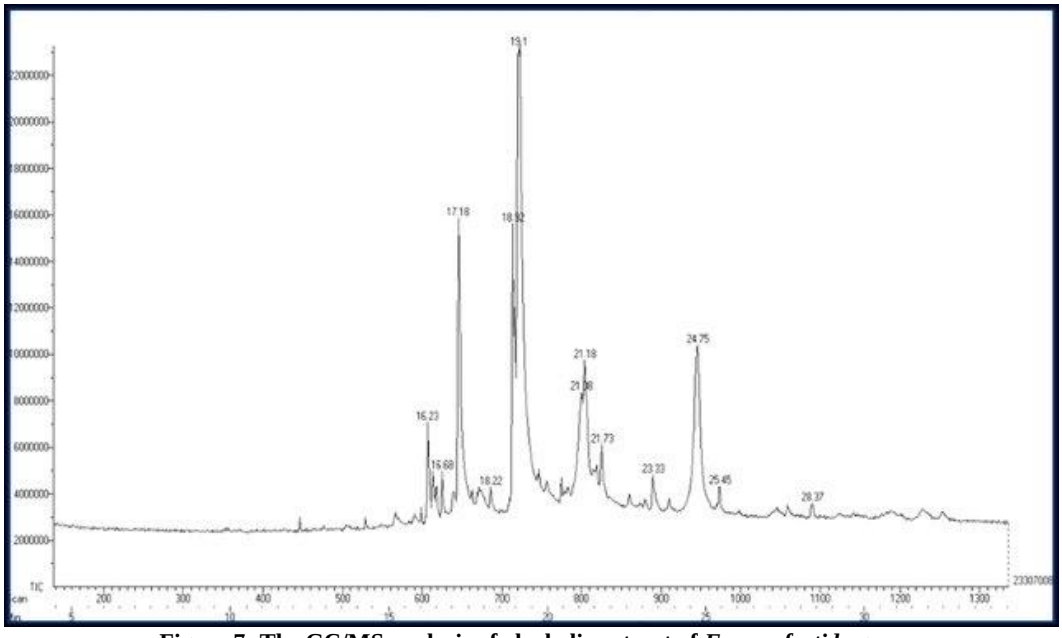

Figure 7: The GC/MS analysis of alcoholic extract of *F. assa-foetida* gum

DISCUSSION

Based on findings of the present study, the 50% and 90% cytotoxicity rates were observed at 50µg/mL and >100µg/mL, respectively for all applied CRC cell lines. According to previous studies, the *F. assa-foetida* L ethanolic extract has exerted significant anticancer effects against Hep-2 cells at 50, 100 and 200µg/mL after 24, 48 and 72hrs of

exposure compared to the L929 normal cells [13]. Hence, the percentage and morphological features of normal cells were not affected by the *F. assa-foetida* L at 24hrs. while cellular rounding was observed after 72 hr. in SW742 cell culture. These differences may be due to the fact that normal and cancerous cells are different in gene expression pathways, membrane ligands and cellular signaling pathways which lead to different responses to the herbal bioactive compounds [14]. The detected morphological change in SW742 cell culture give a signal for potent cellular depression. It was reported that since the attached cells tend to look rounded, this leading to cellular loosing and dis-junctions. Thus become weaker [15, 16]

The anticancer effects of *F. assa-foetida* L backs to various mechanisms such as anti-oxidant features, decrease of DNA and RNA biosynthesis, catalysis of carotenoids, decrease of intracellular sulfhydryl groups, inhibition of endothelial growth factor (mainly by farnesiferol C), inhibition of metallo-proteinase in cancer cells, antimutagenicity, induction of apoptosis and alteration of cellular pathways in cancer cells [17-19]. In addition, lipoxygenase inhibitory and radical scavenging traits of various extracts are considerable. In a study, it was exhibited that anticancer effects of *Ferula* species was not significant against A549 and CH1 cell lines. Indeed, bioactive compounds (confrone, tschimgine and stylosin) facilitated the cisplatin sensitivity by cancer cells which was conducted via inhibition of P-glycoprotein causing reversal of resistance to drug [20, 21]. Notably, the cytotoxic properties of tschimgine and stylosin have been determined against 5637 and MCF-7 cancer cell lines [22]. Interestingly, the cytotoxic activity of umbelliprenin bioactive compound has been higher than that from cisplatin against M4Beu cancer cells [23]. It is worth considering that these compounds contain phenolic constituents and have acted as chemopreventive or chemosensitizer agents [20, 21]. In another study, the *F. assa-foetida* ferulic acid (500µg/mL), essential oil (1 and 10 µl/ml) and oleo-gum-resin exhibited anticancer effects against mouse mammary carcinoma 4T1 cells. Additionally, galbanic acid, another bioactive compound from *F. assa-foetida*, has exerted anticancer effects using human umbilical vein endothelial cell (HUVEC) model via G2/M arrest but not apoptosis [24].

In this study, the gum of *Ferula assa-foetida* most common fractions included oxygen-containing sesquiterpenes (45.25) and sesquiterpenes (30.45%).

Terpenes are natural products consist of various group of secondary metabolites, and they almost abundant in every plant species, recently, there has been substantial progress towards the development of novel Terpenes especially monoterpenes substitute for the prevention and treatment of cancer [25].

It has been reported that sesquiterpenes and their derivatives were highlighted as a potent anticancer agents via several mechanisms such as inhibition of lipid peroxidation, modulating nuclear factor kappa (NF-kB), as well as retarding the formation of both reactive nitrogen and oxygen species [26].

CONCLUSION

It was observed that alcoholic extract of *F. assa-foetida* L gum had anticancer effects against various CRC cell lines including SW742, CT-26, HT-29 and WiDr lines. And the potent concentration against CRC was ranged between 50 and 100µg/Ml. However, more advanced experiments should have carried out in vivo to determine the biologically safe dilution. In addition, the suitable dose and route of administration for the gum extract in order to enter in cancer therapeutic fields.

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