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Potential cytotoxicity and preventive effect of Ferula Assafoetida against early carcinogenic changes in colorectal cancer

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ABSTRACT : Methods: Trypain blue and MTT assay to measure the cytotoxicity and to evaluate the chemopreventive potential of Ferula Assafoetida on colorectal carcinoma cell line, using different Biochemical and Molecular technology.

Results: The results revealed dose dependent inhibition of the viability of Caco- 2cell line, with (IC-50) 342.9 µg/mL. The Ferula Assafoetida treated cells showed significantly, increase caspase-3,8,9 expression, decreased TNF-α expression compared to control. Studies carried out by T.A, GST and MDA were chosen for investigating antioxidant effect as additional mechanism for the chemoprevention explained that a balance between free radicals and antioxidants is necessary for proper physiological function. The inhibitory effect of Ferula Assafoetida on TNF-α as target protein and elucidate its binding site.

Conclusion: Ferula Assafoetida is a strong biological activity, preventive effect against cancer colon and a promising anticancer activity.

Keywords: Ferula Assafoetida, chemoprevention, cytotoxicity and Molecular Bioactivity.

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I. INTRODUCTION

Colorectal cancer (CRC) is defined as any malignant neoplasm arising from the inner wall of the large intestine (colon) or rectum according to CRC facts and figures of the American Cancer Society [1]. It accounts for over 9% of all cancer incidences. It is one of the strongest markers of epidemiological and nutritional transition, since its incidence rates increase as the previously high rates of infection related cancers decline in countries with fast social and economic changes. [2] In Egypt as well, CRC is more common in patients from urban (55%) than rural (45%) areas [3]. However, 21% of patients diagnosed with CRC are under 40 years of age, while in the United States only 2-8% of cases occur in this age group [4] It is unclear if this is linked to a more "westernized" lifestyle and diet in the younger generation. The occurrence of CRC is mainly associated with the incidence of aberrant crypt foci, an earliest neoplastic lesion consists of clusters of mucosal cells with an enlarged thicker layer of epithelia than the surrounding normal crypts that progress into polyps [5]. An adenomatous poly or adenoma – arising from glandular cells- is the most common type [6]. This sequence is considered to be a consequence of the accumulation of multiple genetic alterations in colonic epithelium [7]. The two major well-established genetic pathways to CRC are 1) The chromosome instability (CIS) pathway characterized by accumulation of mutations in key genes controlling the cell cycle [8]. 2) The microsatellite instability pathway (MIP) results from a failure of the mismatch repair system to correct base errors and maintain genomic [9.] Herbal plants are used all over the world in different methods both in allopathic and traditional systems. [10] With the advent of industrial revolution and introduction of modern drugs, using herbal plants were ignored for a specific period of time.[11] But the obstacles on the way of natural compounds studies have recently diminished mostly through using modern techniques. This has resulted in higher interest in using natural compounds in pharmaceutics. [12] According to World Health Organization (WHO) statistics, 80% people all over the world, use traditional treatment13.

Ferula Assafoetida (F. Assafoetida), is an oleo-gum-resin that is extracted from the stems of Ferula plants, which are members of the Umbelliferae family [14]. It is used as a flavouring additives, anticonvulsant and anti-inflammatory medicinal herpes in many countries including Central Asia, particularly West Afghanistan, Iraq, Turkey, Eastern Iran, Europe, and North Africa [15]. F. Assafoetida has many biological functions, especially on gastrointestinal health such as promoting salivary glands secretion, bile acid production, and increased activity of the pancreatic and intestinal digestive enzymes [16,18]. It was also well recognized for its antioxidant [19], anti-diabetic [20], hypotensive, antispasmodic [21], antimicrobial [22,23], and neuroprotective effect [24].

Moreover, many studies reported the potent chemo-preventive effect of F. Assafoetida against the development of different cancers including colon, breast, and cutaneous cancer using experimental animal models [25]. The goal of this study is the evaluate of potential preventive effect of F. Assafoetida and cytotoxicity against early carcinogenic changes in colorectal cancer.

II. Materials and Methods

2 .1 Preparation of plant extract

Ferula assafoetida roots were collected from Saint Catrine, Egypt. The dry gum of root was pulverized into fine powder and stored for further use. The F. Assafoetida was purchased from the Egyptian Company for Medical Herbs, Egypt. The resin was cut with a knife and crushed into small pieces by using a blender. Then, the resin was soaked in the extraction material either absolute ethanol, methanol, or water. The F. Assafoetida was extracted using the cold maceration method, where the resin was macerated in the solvent for three days on a shaker at room temperature. Then, the resin was filtered using 101 Double Rings filter paper. The diluted liquid extract was concentrated with a rotary evaporator to obtain a viscous liquid. This liquid was finally dried in the dryer for one day to remove the remaining traces of solvent.

a_ Methanolic extract preparation

About 100 gm of dried oleo-resin-gum was separately extracted with methanol at room temperature for 72 h, where this procedure was repeated 3 times. The methanolic extract was concentrated under reduced pressure. The extract was then washed with 100 mL hexane for three times. Then it was stored at 4 °C in sealed vials until usage.

b_ Ethanol extract preparation

For the preparation of ethanolic extract, more than 100 gm of dried oleo-resin-gum was pulverized. Then 100 gm of the obtained powder was soaked in 1000 ml of 70% ethanol for 48 h at room temperature. The solvent was filtered with Whatman filter paper (grade 40) and soaked in 70% ethanol again. This process was conducted four times. The solvent was evaporated using rotary evaporator and the collected extract was freeze dried and finally stored in the refrigerator at 4 °C until needed.

c_ Water extract preparation

One hundred grams (100g) of F. Assafoetida was soaked in 600 ml distilled water for 72 h at room temperature. Then, the extract was filtered and dried at 4°C using a rotary evaporator.

2.2 Determination of Total Flavonoid Content in *F. Assafoetida*

Total flavonoid content of the F. Assafoetida methanolic extract was detected by the classical Aluminium chloride ($AlCl_3$) colorimetric method [36]. In brief, 20 µl of 1% $AlCl_3$ dissolved in methanol was mixed with the same volume of the F. Assafoetida. After incubation for 10 minutes, the absorption was detected at 490 nm against a blank sample formed of the same mixture without $AlCl_3$. A standard calibration curve was plotted using different concentrations of quercetin (0–250 µg/mL), where the total flavonoids were measured as mg quercetin equivalent (QE)/g dry weight (DW).

2. 3 Determination of Total Phenolic Compounds in F. Assafoetida

Total phenolics amount of the F. Assafoetida methanolic extract was assessed using Folin-Ciocalteu method [26]. Briefly, 100 µl sample of F. Assafoetida methanolic extract was mixed with 800 µl Na_2CO_3 (700 mM) and 200 µl of 10% Folin–Ciocalteu. After incubation for two hours at 25°C, the absorbance was measured at 765 nm. The phenolic content was expressed as gallic acid equivalent (GAE)/g DW according to the standard calibration curve of gallic acid.

2 .4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC/MS analysis of F. Assafoetida extract was performed using Agilent Technologies GC-MS 7890A spectrophotometer (Agilent, USA), equipped with autoinjector (Agilent 7693A Automatic liquid sampler). The mass selective detector (5975 C) was operated in full scan mode. The ionization source was supplied with a voltage at 70 eV. The GC was fused with silica capillary column; Hewlett-Packard 5MS (30 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was held at 80°C for two min and increased to 300°C isothermally over 20 min. Helium was used as carrier gas with flow rate of 1.5 mL/min. Injector temperature was adjusted at 280°C. The injected volume of F. Assafoetida extract was prepared at One µl and was analysed by GC twice.

The separated sample components were identified by comparing their mass spectra with those recorded standards data base at the National Institute of Oceanography and Fisheries, Alexandria University Egypt.

Maintenance of The Caco Cell Line 2 .5

The Caco-2 cells were obtained from the American Type Culture Collection ATCC, Manassas, VA, USA). The cells were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing in RPMI-1640 medium (RPMI-1640, Sigma–Aldrich, USA), supplemented with antibiotic-free 10% fetal bovine serum (FBS, Sigma, USA), 100 U/ml penicillin, and 2- mg/ml streptomycin. The Caco-2 cells were sub cultivated after trypsinization (Trypsin-EDTA, Cambrex Bioscience Verviers, Belgium) once or twice per week and re-suspended in a complete medium in a 1:5 split ratio to maintain it in the exponential growth phase. They were maintained as a monolayer in T75 cell culture flasks with filter screw caps (TPP, Trasadingen, Switzerland) at 37 °C in a humidified 5% CO2 incubator Revco, GS laboratory equipment, RCO 3000 TVBB, USA).

2 .6 Cytotoxicity Assay

The cell Viability and cytotoxicity of all extracts were done utilizing the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide thiazolyl (MTT) assay [38]. Caco cells were seeded ($5x10^3$/ well) in 96-well flat-bottomed microlitre plates in RPMI-1640 media supplemented with FBS and incubated overnight at 37 °C in a humidified 5% CO_2 incubator. The cells were placed in a fresh medium containing serial dilutions of NaBT (0.65-10 mg/mL), ethanolic, methanolic, and water extracts of F. Assafoetida (100-1600 µg/mL). The cells were incubated for 24 hours at 37 °C and 5% CO2. A 10 µl of MTT solution (5 mg/ml in PBS) was added to each well and Shaked for 5 minutes, then the mixture was incubated in the dark for 4 hours. The medium was then removed after centrifugation and 100 µl of isopropanol was added for the assessment of cell viability. The absorbance was measured by the ELISA reader (TECAN SunriseTM, Germany) at 570 nm. Cell viability was measured by dividing the absorbance of the treated cells by that of the untreated cell. All experiments were done in triplicate. The 50% inhibitory concentration (IC_{50}) was calculated through the sigmoidal dose response curve-fitting equation using GraphPad Prism 8.4.2 (GraphPad Software, La Jolla, CA, USA), where the least IC50 was chosen.

2 .7 Cell treatment

The Caco cells ($5x10^5$ cells/well) were divided into three groups; the first group which treated with the methanolic extract of F. Assafoetida (342.9 µg/mL), the second which treated with the NaBT (3.3 mg/ml), and the untreated cells were considered as a control group. All group of cells were incubated for 48 hours in humidified 5% CO_2 incubator at 37 C for further analysis.

2 .8 Cell Cycle Analysis

The cell cycle analysis was determined using Cytell ™ cell cycle kit (GE Healthcare Japan, Tokyo, Japan), according to the manufacture instructions. The cell groups were incubated for 24 hours in a 5% CO_2 incubator at 37°C. The DNA content and the cell cycle phases were detected using Cytell™ cell imaging system (GE Healthcare Japan).

2 .9 Apoptosis Assay

Apoptosis assay was determined using the Invitrogen™ Alexa Fluor™ 488 annexin V/Dead Cell Apoptosis Kit (Invitrogen™, ThermoFisher Scientific, USA) according to the manufacturer's instruction. Briefly, all groups of cells (cells treated with F. Assafoetida, and untreated cells) were washed with cold PBS and centrifuged. Then cells were resuspended in 100 µL annexin-binding buffer (1×10^6 cells/mL), five µL Alexa Fluor 488 annexin V, and one µL (100 µg/mL) propidium iodide (PI) working solution. The cell groups were incubated for 15 minutes at room temperature, then 400 µL 1X annexin-binding buffer was added and the cells were kept on ice for detection of apoptotic cells using Cytell TM cell imaging system (GE Healthcare Japan).

2 .10 DNA fragmentation Assessment

Assessment of DNA fragmentation was performed based on the detection of DNA ladder appearance at late stage of apoptosis as described by (Elmore et al., 2007). DNA was extracted from untreated Caco cells (as a control) and those treated with F. Assafoetida. The DNA was prepared at a concentration of 300-600 ng/µL. Then it was run in pre-stained agarose gel electrophoresis 1.5%. The DNA was visualized and photographed under ultraviolet illumination [27].

2 .11 Antioxidant Activity

The total antioxidant (TA), glutathione reductase (GSH), and Malondialdehyde (MDA) concentrations in the treated and untreated Caco cells were assessed using Biodiagnostic kits (Cat.no. TA 25 13, GR25 11, and MD 25 29; respectively, Cairo, Egypt). According to the manufacture instructions. The TA, GSH, and MDA concentrations were measured calorimetrically at 505 nm, 405 nm, and 534 nm: respectively, using a spectrophotometer (UV-2505, LaboMed, inc. USA).

2 .12 Statistical Analysis

Data were analysed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). The results were expressed as mean and standard deviation (SD) according to the Kolmogorov–Smirnov and Shapiro– Wilk normality tests. Comparison between variables was done using the student's t-test and the one-way analysis of

variance (ANOVA) with post hoc Tukey's test. Cytotoxicity curves were done using GraphPad Prism 8.4.2. (San Diego, CA, USA). All tests were performed as two-tailed, and the significant level was adjusted at p-value ≤ 0.05.

III. Results

3 .1 Cytotoxicity Assay of F. Assafoetida on Caco cells

The cytotoxicity effect of F. Assafoetida on Caco cells was determined according to the concentration-**DEPENDENT** decrease in the growth rate of the cells. Doses that inhibited cell growth to 50% (IC_{50}) were 342.9 µg/mL for methanolic extract, of F. Assafoetida.

3 .2 Determination of The Total Phenolic and Flavonoid Compounds of The F. Assafoetida Methanolic Extract

The total phenolic and flavonoid content present in the methanolic extract of F. Assafoetida extract were 3.101 (g/100g) and 0.797 (g/100gm), respectively**.**

3 .3 Phytochemical Analysis of The Methanolic Extract of F. Assafoetida Using GC-MS

GC-MS analysis of the methanolic extract of F. Assafoetida revealed the presence of 26 phytochemical compounds. The identification of the phytochemical compounds was based on the peak area, retention time, and the molecular formula (**Table 1, Figure 1B**). The major compounds were coumarins (31.17%), Torreyol (δ-Cadinol) (7.63%), L-ascorbic acid 6-stearate (7.46%), 2-Mercapto-3,4-dimethyl-2,3dihydrothiophene (7.29%), Cadinene (4.22%), Aristolene (3.54%), 1-heptatriacotanol (3.18%), Vanillin (3.07%), beta-guaiene (3.03%), Dotriacontane (2.69%), Octadecanoic acid,4-hydroxy-methylester (2.45%), Dasycarpidan-1-methanol acetate (2.32%), 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene (2.29%), 3,4 Dimethyl-thiophene (2.28%), 2,4,4-Trimethyl-3-(3-methylbuta-1,3-dienyl) cyclohexanone (2.27%), Tetracosane 2,6,10,15,19,23-hexamethyl (2.13%), phenyl aziridine carboxylate (1.68%), isolongifolene (1.61%), Rhodopin (1.54%), Styrene (1.48%), N,N,N-Trimethyl-1,4-phenylenediamine (1.46%), 1-chloro Octadecane (1.23%), 3-Oxo-20-methyl-11-alpha-hydroxyconanine-1,4-diene (1%), 10,12-Tricosadiynoic acid methyl ester (1%), and Bicyclo{4,4,0}dec-1-ene, 2-isopropyle-5-methyl-9-methylene (0.92%).

3 .4 Effect of F. Assafoetida on The Cell Cycle

There was a significant increase in the cell number treated with F. Assafoetida (53.85±0.03%), in the sub-G1 phase, compared to the untreated control cells (0.78±0.03%, **p<0.001**). While there was a significant deference in the number of cells in the other cycle phases for those treated with F. Assafoetida (G0/G1 phase (29.27±0.03%), S phase: 12.76±0.05%, G2/M: 2.9±0.04%, and >4n: 1.17±.03%, **p<0.001**), S phase: 10.65±0.01%, G2/M: 8.7±0.01%, and >4n: 0.17±0.20%, **p<0.001**), compared to the control cells (G0/G1 phase (67.41±0.02 %), S phase: 30.73±0.11%, G2/M: 0.79±.01%, and >4n: 0.24±0.01%, **Figure 2**).

3 .5 Effect of F. Assafoetida on Apoptosis

Apoptosis was significantly increased in the Caco cells treated with F. Assafoetida (49.1± 0.14 %) in comparison to untreated control cells (11.1±0.02 %, **p<0.001**). While the number of live cells in each group represented 50.9±0.12 % in the Caco cell treated with F. Assafoetida compared to 88.9±0.10 % in untreated control cells (**p<0.001**, **Figure 3**).

3.6 DNA fragmentation Assessment

The results of DNA fragmentation analysis showed complete DNA degradation in Caco cells treated with the methanolic extract of F. Assafoetida with the dose of IC50 (342.9 µg/mL). While at the dose of half and forth of IC50, there were slight DNA degradation when compared with untreated cells (**Figure 4A-C**).

3.7 Role of F. Assafoetida on Cellular Oxidative Stress

There was a significant increase in the total antioxidant concentration in Caco cells treated with F. Assafoetida (0.625±0.009 nmol/ml) in comparison to untreated control cells (0.138±0.001 nmol/ml, **p<0.001, Figure 5A**). Additionally, there was a significant increase in the GSH concentration in Caco cells after treatment with Ferula Assafoetida (0.036±0.005 nmol/ml), in comparison to the untreated control cells (0.019±0.001 nmol/ml, **p<0.001, Figure 5B)**. On the other hand, there was a significant decrease in the MDA concentration in Caco cells treated with F. Assafoetida (0.370±0.004 nmol/ml) in comparison to the untreated control cells (0.886±0.005 nmol/ml, **Figure 5C**).

Figures' legends

Figure 1: A) Cytotoxicity assay of the methanol, ethanol, and water extract of F. Assafoetida, and on Caco cells using MTT assay. B) The GC-MS chromatogram of the methanolic extract of F. Assafoetida

Figure 2: Cell cycle analysis of A) untreated control Caco cells, B) Caco cells treated with Na. butyrate, and C) Caco cells treated with F. Assafoetida. The cell cycle analysis was detected with Cytell Cell Imaging system.

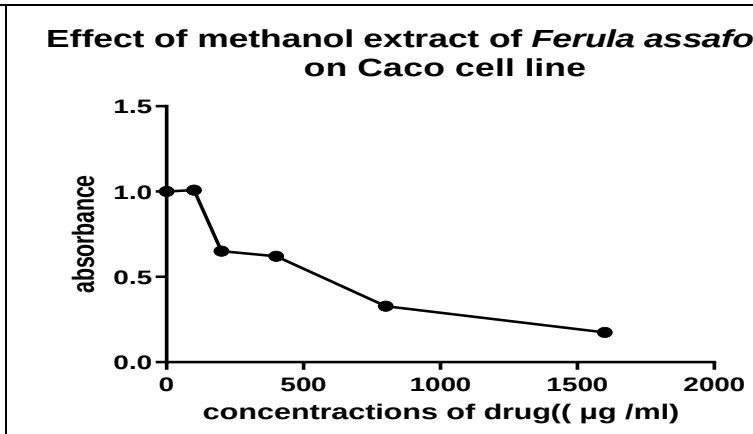
Figure 3: Histogram charts showed the percentage of (A) live cells and (B) apoptotic cells in: C) Caco cells without treatment, D) Caco cells treated with Na. butyrate, and E) Caco cells treated with F. Assafoetida. Apoptosis was detected with Cytell Cell Imaging system.

* Variables with different letters ate significantly different (p<0.05).

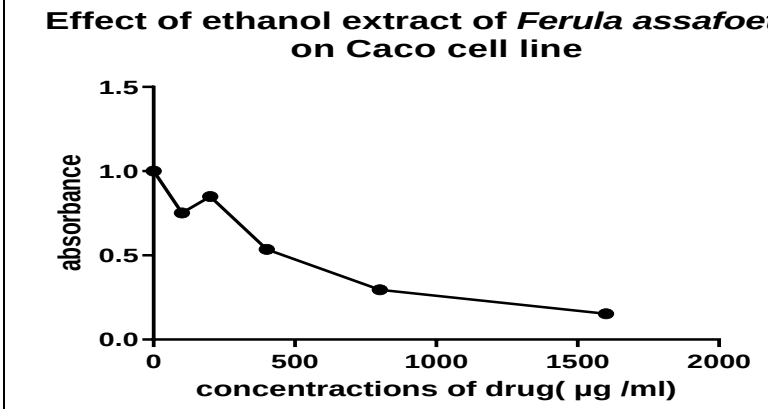
Figure 4: Effect of F. Assafoetida on Cellular Oxidative Stress A) total antioxidant, B) Malondialdehyde (MDA), and glutathione reductase (GSH) concentrations in Caco cells treated with F. Assafoetida, NaBT against untreated control Caco cells. **C**) ALP activity in Caco cells treated with Ferula Assafoetida against untreated control Caco cells. *Variables with different letters are statistically different.

Figure 5: A) Microscopic examination of untreated Caco cells and B) those treated with F. Assafoetida showing the cytotoxic effect of F. Assafoetida on the cells (magnification 20X). C) Electropherogram for DNA fragmentation of Caco cells treated with methanol extracts of F. Assafoetida at conc. (1/4 IC50, 1/2 IC50, and the IC50) µg/mL. M: DNA marker, C: control.

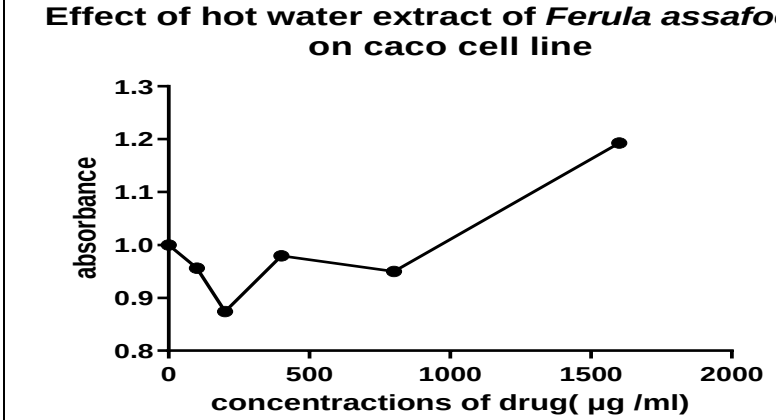
A



B



C



Cell viability of Caco-2 cell line treated with A) methanol extract, B) Ethanol extract, and C) water extract of F. *assafetida* at concentration range of (100 µg/ml -1600 µg/ml) .

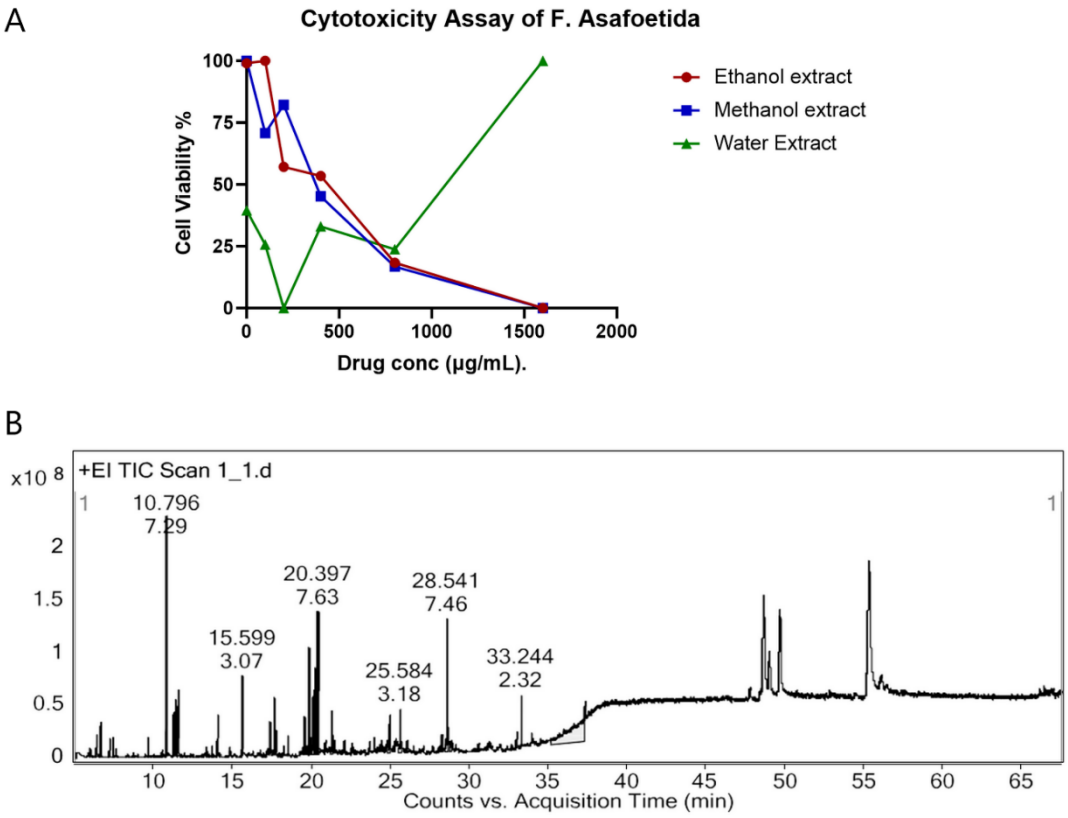
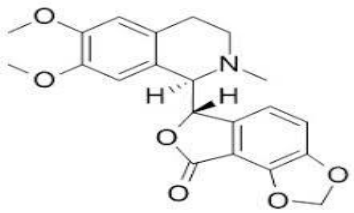
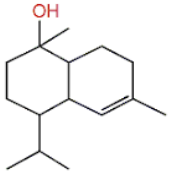
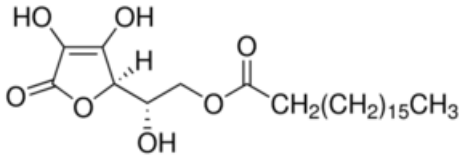
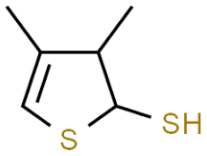
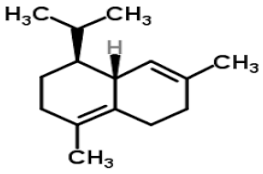
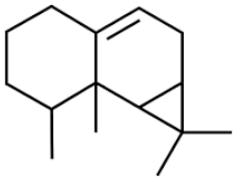
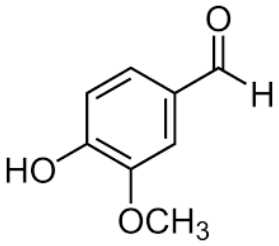
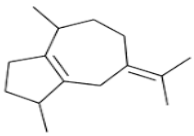
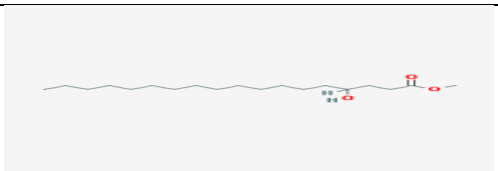
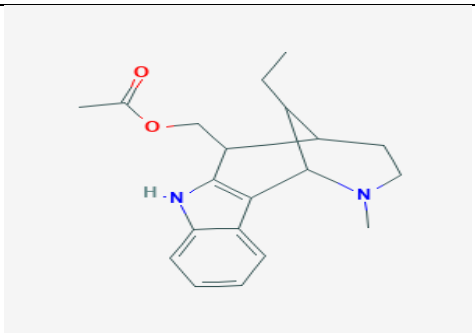
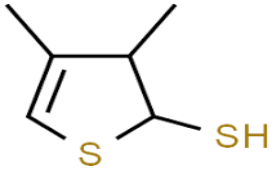
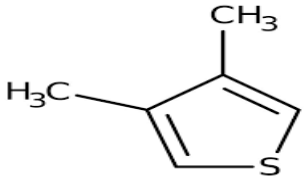
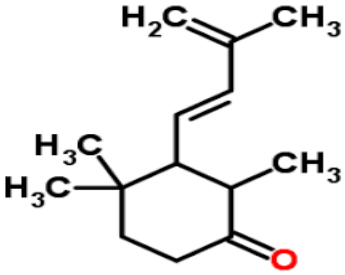
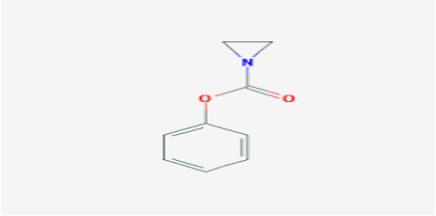
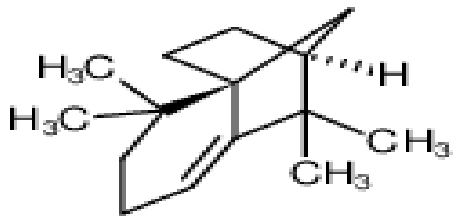
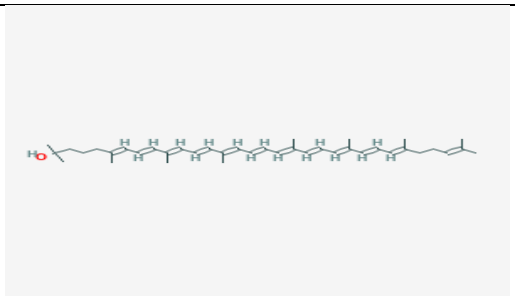
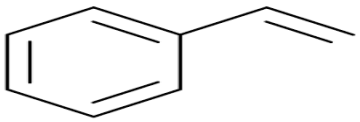
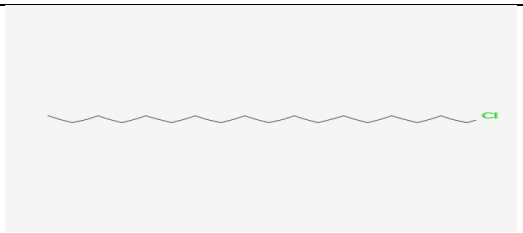
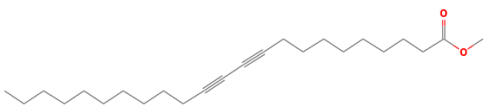


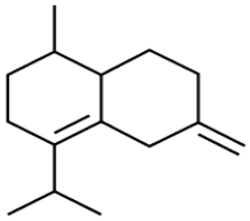
Table (1): Phytochemical compounds identified in methanolic extract of *F. Assafoetida*

	Compound	**Amount acc to area sum%**	**Chemical structure**
1.	Corlumine	31.17% 1.04%	
2.	Torreyl	7.63%	

3.	L-ascorbic acid ,6-stearate	7.46%	
4.	2-Mercapto-3,4-dimethyl-2,3dihydrothiophene	7.29%	
5.	Cadinene	4.22%	
6.	Aristolene	3.54%	
7.	1-heptatriacotanol	3.18%	$CH_3(CH_2)_{36}$ OH
8.	Vanillin (Benzaldhyde,4-hydroxy-3-methoxy)	3.07%	
9.	beta-guaiene	(3.03%),	
10.	Dotriacontane	(2.69%),	$CH_3(CH_2)_{30}CH_3$

11.	Octadecanoic acid,4-hydroxy-methyl ester	(2.45%),	
12.	Dasycarpidan-1-methanol, acetate	(2.32%)	
13.	2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene	(2.29%)	
14.	3,4Dimethylthiophene	(2.28%),	
15.	2,4,4-Trimethyl-3-(3-methylbuta-1,3-dienyl) cyclohexanone	2.27%	
16.	Tetracosan 2,6,10,15,19,23-hexamethyl(squalane)	2.13%	
17.	phenyl aziridine carboxylate	1.68%	

18.	Isolongifolene	(1.61%)	
19.	Rhodopin (psi.,psi.-Carotene,1,2-dihydro-1-hydroxy)	1.54%	
20.	Styrene	1.48%	
21.	N, N,N-Trimethyl-1,4-phenylenediamine	1.46%	
22.	1-chloro, Octadecane	(1.23%)	
23.	Corlumine	1.04%	Not found on the table
24.	3-Oxo-20-methyl-11-alpha-hydroxyconanine-1,4-diene	1%	C22H31NO2
25.	10,12-Tricosadiynoic acid methyl ester	1%	

26.	Bicyclo {4,4,0}dec-1-ene,2-isopropyle-5-methyl-9-methylene	0.92%	

	Count of nuclei	<2n	G_0/G_1	S	G_2/M	>4n
-ve control	1246	0.79%	67.41%	30.78%	0.79%	0.24%
F. assafoetida	1442	53.88%	29.26%	12.76%	2.91%	1.18%

Table (2.) Cell cycle analysis of Caco-2 cells treated with the IC_{50} concentration of of F. assafoetida extract, and the control untreated cells.

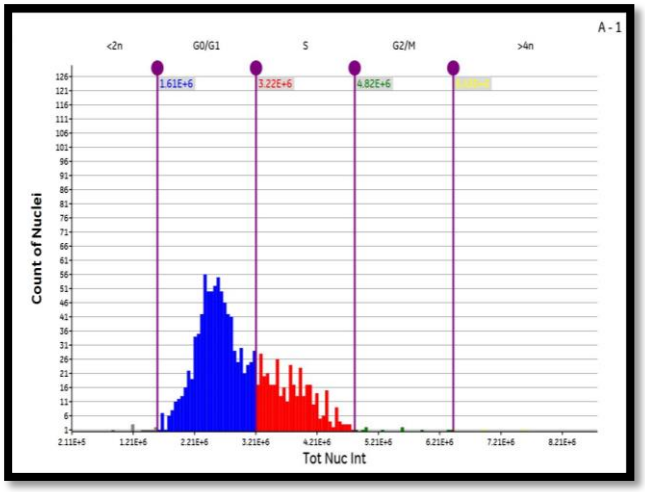


Figure (a): Histogram graph show cell cycle analysis for negative control of (untreated Caco-2 cells).

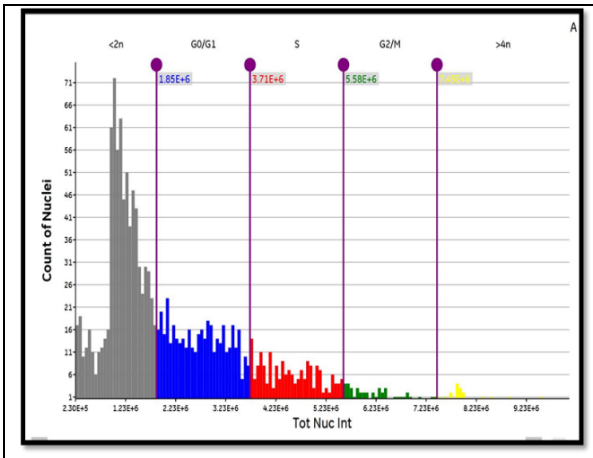


Fig.(b) Histogram graph show cell cycle analysis of Caco-2 cell treated with methanol extract of *F. assafoetida*

Cell cycle analysis of Caco-2 cells treated with the IC_{50} of F. assafoetida incubated at 37 ºC in a 5% CO2 incubator for 24 h analyzed using a CytellTM cell imaging system.

Table (3): The apoptotic effect of *F. assafoetida* methanol extract on Caco-2 cells. Data presents as means ± SD

Well	Live Cells (%)	Apoptotic Cells (%)
A-8 (control)	88.88 ±0.1	11.12 ± 0.01
A-12 (*F. assafoetida*)	50.92 ±0.1	49.08 ±0.1

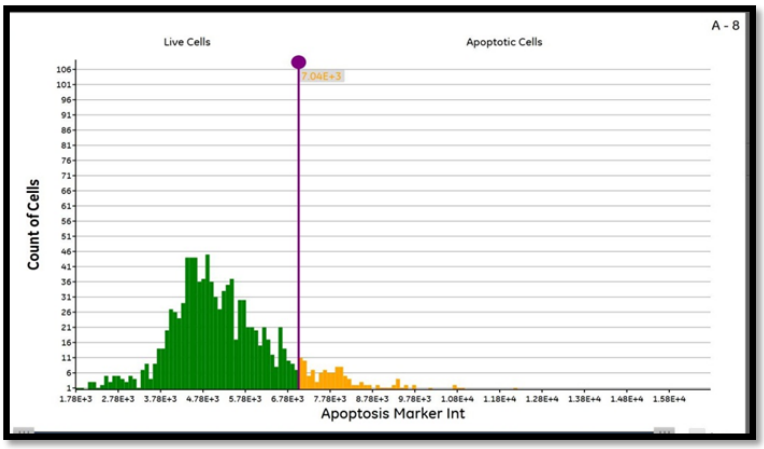


Figure (c): Histogram chart showed the percentage apoptotic cells of Caco untreatment cells (Control).

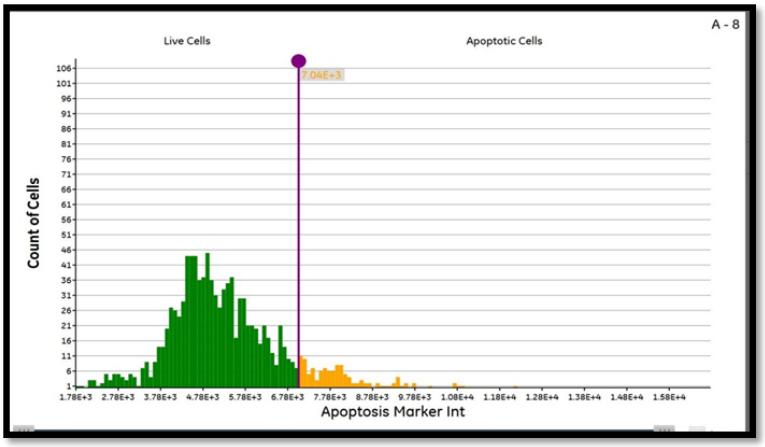


Figure d: Histogram charts showed the effects of Na. butrate on apoptosis of Caco cell line. The cells treated at concentration 3.3 µg/ml. Apoptosis was detected with Cytell Cell Imaging system.

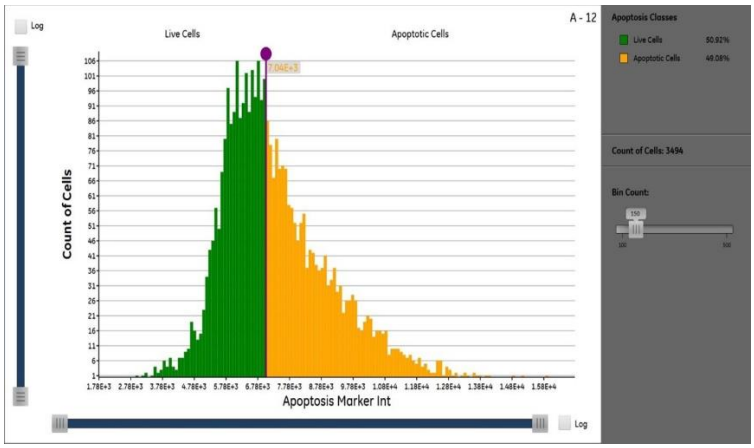
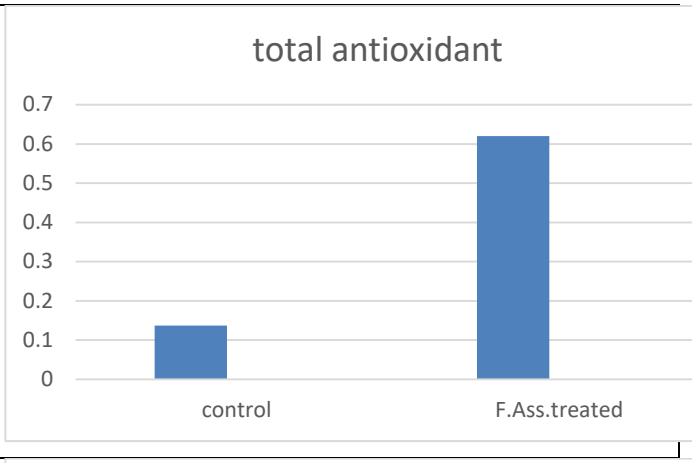


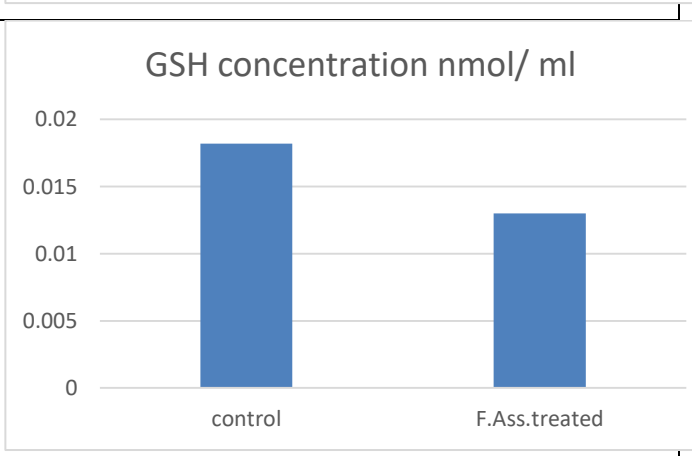
Figure d: Histogram charts showed the effects of *Ferula* methanol extract on apoptosis of Caco cell line. Apoptosis was detected with Cytell Cell Imaging system.

Results for the antioxidant activity of cells treated with F. *assafoetida extract*

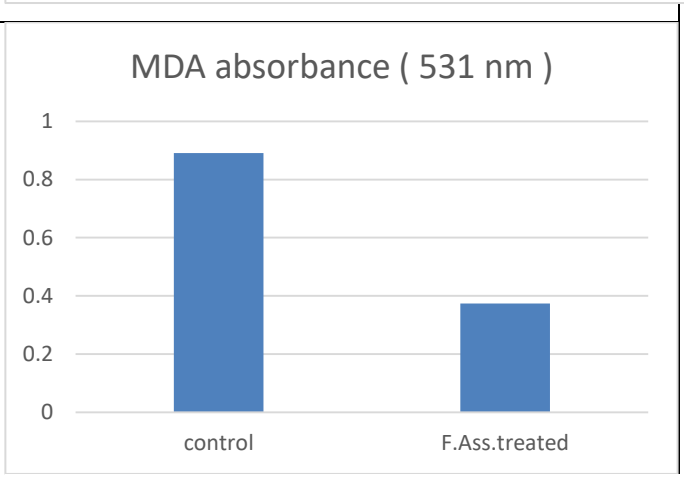
Sample	Total antioxidant absorbance
Control	0,137
F. assafoetida	0,616

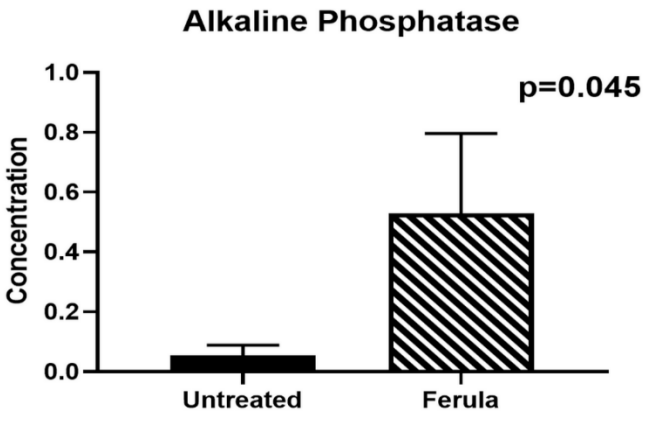


Sample	GSH concentration nmol/ ml
Control	0.0182
F. assafoetida	0.0126



Sample	MDA absorbance at (531 nm)
Control	0.891 - 0.881
F. assafoetida	0.374 - 0.3660





Sample	0.05±0.03
F. assafoetida	0.53±0.26
p value	0.045

Results of DNA fragmentation of Caco2 cells

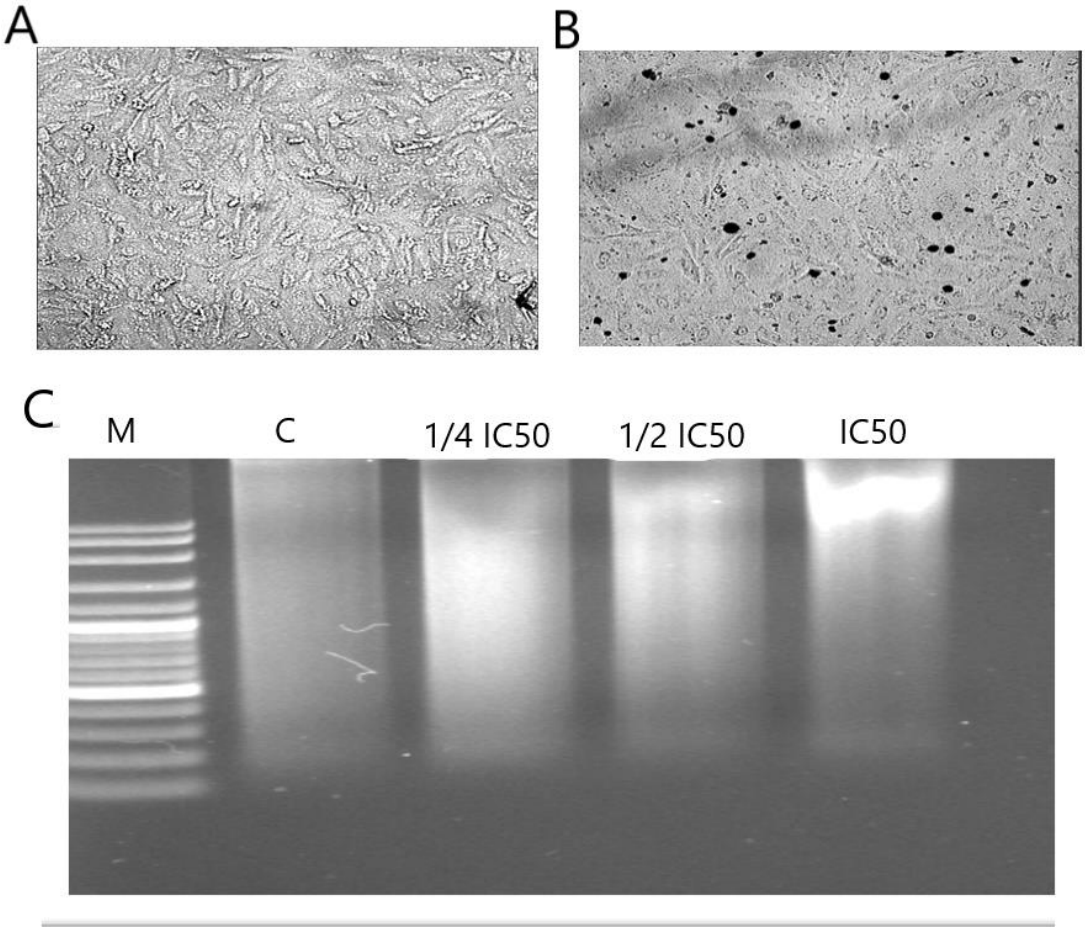


Figure: Histogram charts showed the effects of *F. assafoetida* methanol extract on apoptosis of Caco cell line.

IV. Discussion

The CRC is a major health problem with increasing mortality rates worldwide. This is due to the presence of the dedifferentiated colon cancer cells which are responsible for the emergence of disease recurrence and resistance to the conventional chemotherapeutic drugs [28,29]. Therefore, new strategies aiming at modulating the differentiation capabilities of cancer cells can be a promising line of successful treatment for colon cancer patients [30]. The current study showed that F. Assafoetida has a potential anticancer activity through reprograming and induction of colon cancer cell differentiation into functioning normal calls. Chen and his colleagues found that ferulic acid (FA; a component of F. Assafoetida) inhibited colon cancer proliferation both in vitro and in BALB/c mice CRC model through the stimulation of BCL-2 and BAX required for the apoptosis pathway [31].

Therefore, we used it to assess the efficacy of F. Assafoetida in colon cancer cell differentiation. The present data showed that the number of Caco cells treated with F. Assafoetida arrested at the sub-G1 phase that

represents the cells undergoing apoptosis were significantly increased, untreated control cells. Also, there was a significant decrease in the cell number treated with F. Assafoetida in the S. (DNA synthesis) phase, compared to the control cells. Incomparable with these findings, Janicke et al concluded that ferulic acid exerts antiproliferative effects on Caco-2 cells through upregulating RABGAP1 and CEP2 gene expression, which were implicated in centrosome assembly, as well as the gene involved in S-phase checkpoint protein SMC1L1 [32]. While Bagheri et al found that F. Assafoetida produces cell cycle arrest at G0/G1 phase that was responsible for its neuroprotective effect [33].

The current results showed that the efficacy of F. Assafoetida to induce apoptosis in Caco cells was significantly higher than compared to the untreated control cells. Additionally, there was a complete DNA degradation (which is a histological sign of apoptosis) in the Caco2 cells treated with the dose of IC50 of F. Assafoetida. These data are consistent with that of Elarabany et al [34], who informed that F. Assafoetida exhibited an antitumor effect against colon cancer cells through decreasing cell viability and induction of apoptosis. Zhang et al, also reported that the antiproliferative effect of ferulic acid on breast cancer cells was accomplished through the induction of apoptosis [35]. Similarly, Efati et al, found that F. Assafoetida L. extract zinc nanoparticles, exerts antiapoptotic and antioxidant functions on CRC and breast cancer cell lines through upregulating Bax and downregulating BCL-2 gene expression [36].

Regarding the impact of F. Assafoetida on Cellular Oxidative Stress, the present data showed that F. Assafoetida induced a significant increase in the total antioxidant concentration in Caco cells in comparison to those treated with untreated control cells. Also, it exhibited a significant increase in the GSH concentration in Caco cells in comparison to the untreated control cells. While it induced a significant decrease in MDA concentration in Caco cells compared to the untreated control cells.

Moreover, the phytochemical analysis of the methanolic extract of F. Assafoetida revealed a large amount of phenolic and flavonoid content which proved to have antioxidant, anti-inflammatory, and antitumor effects [34, 35,36]. This was illustrated by the presence of high amount of some beneficial components including e.g. coumarin (31.17%), L-ascorbic acid 6-stearate (7.46%), Cadinene (4.22%), as well as many sulfur-containing compounds. All these components have been reported to exhibit potent antioxidant, antimicrobial, neuroprotective, and anticancer functions [34,35, 56,37].

In conclusion, the present study provided evidence that F. Assafoetida could be important implications for cancer therapy through arresting cells in the sub-G1 phase, inducing cytotoxicity, DNA fragmentation, and apoptosis in the cancer cells compared to untreated control cells. Moreover, F. Assafoetida has a protective impact against the cellular oxidative stress revealed by the significant increase in the TA concentration and GSH level, while a significant decrease in the MDA levels in the Caco cells compared to the untreated control cells. Therefore, more research should be directed to F. Assafoetida as a useful, efficient, inexpensive, and novel therapeutic medicinal herps for colon cancer patients.

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