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Original article

Apoptotic effects of *Corchorus olitorius* L. leaf extracts in colon adenocarcinoma cell lines

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Summary. *Corchorus olitorius* L. is a highly consumed plant in Cyprus and other Eastern Mediterranean countries and known as a medicinal food in many cultures. The aim of the study is to determine *in vitro* anticancer and apoptotic induction effects of dichloromethane (DCM) and aqueous *Corchorus olitorius* L. leaf extracts in primary (Colo-320) and metastatic (Colo-741) colon adenocarcinoma cell lines.. Cell growth and cytotoxicity were measured with MTT assay with five different concentrations of extracts in Colo-320 and Colo-741 cell lines. Apoptotic activities of *Corchorus olitorius* L. were investigated by immunocytochemistry using antibodies directed against to caspase-3, cytochrome-c and FasLigand (FasL). TUNEL assay was used to detect DNA fragmentation in both cell lines. Both DCM and aqueous phase of extracts at 50 μg/ml concentration were more effective at inhibiting Colo-320 and Colo-741 cell growth when compared with other dilutions. The number of TUNEL positive cells was significantly higher in Colo-741 cells when compared with both control group and Colo-320 cell line. DCM phase extract significantly increased caspase-3 immunoreactivity while aqueous phase extract significantly increased cytochrome-c and FasLigand (FasL) immunoreactivities in Colo-320 cell lines. Both extracts were able to significantly increase caspase-3, cytochrome-c and FasL immunoreactivity in Colo-741 cells showing that both apoptotic pathways were triggered. Overall, *Corchorus olitorius* L. extracts induced apoptosis in both cancer cell lines while being more effective in metastatic colon adenocarcinoma cell lines suggesting that the extracts might have potential anticancer effects and possibility to be used as precursor to phytomedicinal colon cancer treatment as oppose to chemotherapy.

Key words: *Corchorus olitorius*, colon cancer, apoptosis, plant extract

Introduction

Corchorus olitorius L. is a plant which belongs to Tiliaceae family in botany. It is a dark green leafy vegetable, which is highly consumed in Cyprus, Eastern Mediterranean and Middle Eastern countries (1–3). *Corchorus olitorius* L. is known as a medicinal food in many cultures and has therapeutic effects such as being sedative, diuretic and laxative (4). In addition, according to folk remedy, *Corchorus olitorius* L. is beneficial for treatment of dysuria, cystitis and gonorrhoea (5).

Corchorus olitorius L. leaves contain macro and micronutrients as well as polyphenols (6–8). It is also found to be rich in antioxidant compounds such as caffeoylquinic acid, quercetin glycosides, vitamin E and omega-3 fatty acids (6–9). *In vivo* and *in vitro* studies had shown that *Corchorus olitorius* L. leaf extract has antioxidant properties and reduces oxidative stress due to its rich phytochemical content (7,8). Furthermore, ionone glycosides in *Corchorus olitorius* L. had shown to suppress histamine release and corchorifatty acids inhibited lipopolysaccharide-induced nitric oxide (NO) production in cultured mouse peritoneal macrophages (10).

It is estimated that colorectal cancer is the third most cause of cancer mortality in the world (11). The phytochemical constituents of ethnomedicinal edible plants in the maintenance of health and protection from cancer are also of interest in prevention and treatment

of cancer (12,13). The use of traditional plants as anticancer drugs, by isolating potential bioactive molecules, is becoming a more popular side method than chemotherapy. (13). The previous studies had shown potential anticancer effect of *Corchorus olitorius* L. extract on human multiple myeloma and hepatocellular carcinoma cells (2,14). The specific effects of *Corchorus olitorius* L. extract in cancer cell viability and apoptosis in colon carcinoma cells still remains undefined. To the best of our knowledge, there are no reports addressing the effect of *Corchorus olitorius* L. extract in colon cancer.

Primary and metastatic forms of colon cancers might have different mutations. Some of the prominent mutations in primary colorectal cancers are also found in subsequent metastatic tumours. However, there is also discordance between the primary tumour mutations and metastatic tumour mutations (15). Metastatic tumours have more p53, KRAS and BRAF mutations, which induce cell proliferation, differentiation and evasion of apoptosis (16,17). In addition, overexpression of growth factors might be a cause of transformation of primary into metastatic colon cancer (16).

Apoptosis plays key a role in final decision of cancer cell's fate. It is necessary for multicellular systems where the body gets rid of deteriorated cells that might transform into malignant cancerous formations (18,19). Activation of intrinsic pathway requires internal stimuli which increases mitochondrial permeability and causes release of cytochrome-c into the cytosol. The release of cytochrome-c triggers apoptosome, that activates caspase 9/3 signalling cascade and therefore initiates the final step of apoptosis (18,20). On the other hand, in extrinsic pathway, attachment of an external stimulus to death receptor, FasLigand (FasL) initiates apoptosis by causing formation of death inducing signalling complex (DISC). This process triggers the start of caspase activities including caspase 8 and 10 and finally the last caspase prior to apoptosis, caspase 3 (18,20,21).

The aim of the study is to determine *in vitro* apoptotic induction effects of dichloromethane (DCM) (lipophilic) and aqueous (hydrophilic) *Corchorus olitorius* L. leaf extracts in primary (Colo-320) and metastatic (Colo-741) colon adenocarcinoma cell lines. In addition, we aimed to determine the molecular apoptotic pathway that is triggered after treatment with both extracts in both cell lines.

Materials and Methods

Plant material and extraction

Mature *Corchorus olitorius* L. leaves were collected from Kyrenia, Cyprus in July 2016. The collected plant sample was registered with Near East Herbarium at Near East University under the Herbarium number 6904. The dry leaves of *Corchorus olitorius* L. (100 g) were powdered (Waring Commercial Blender, United States of America, USA) and extracted with 80% (800 ml) ethanol while incubated overnight at room temperature with occasional stirring. The extract was vacuum filtered and concentrated to 200 ml by rotary evaporator (BUCHI Rotavapor R-210). Concentrated aqueous phase was then extracted with DCM to remove lipophilic compounds by using separation funnel (x3). DCM extract was evaporated to dryness and kept over $CaCl_2$ desiccator for 48 h. The aqueous phase was concentrated to its half and lyophilized (Christ Alpha 1-4 LD Plus, Germany) with freeze-dryer. The total yields for DCM and aqueous phase extracts were 3.7% and 14.8% respectively.

Cell line and cell culture

Cell lines, Colo-320 (ATCC: CCL-220.1) and Colo-741 (ECACC: 93052621) were maintained in RPMI-1640 medium (Biochrom, FG 1215), 10% heat inactivated fetal bovine serum (FBS) (Capricorn Scientific, FBS-11B), 1% penicillin-streptomycin (Biochrom, A2213) and 1% glutamine (EMD Millipore, K0282). Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2. As the cultured cells reached confluency state, they were sub-cultured using 0.25% trypsin-EDTA solution (Biochrom, L 2143).

Cell viability and growth assay

DCM and aqueous phase extracts were dissolved with dimethylsulfoxide (DMSO, Sigma-Aldrich) to 100 mg/ml. The extracts were further diluted in culture medium (5 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml and 100 µg/ml). The final concentration of DMSO in cell lines was less than 0.05%. Colo-320 and Colo-741 cells were collected, suspended in medium and seeded in 96-well culture dishes at a density of 5 x 10^4/ml cells in each well with 100 µl medium. Extract dilutions were triplicated for both DCM and aqueous phases. Both cell lines were incubated for 24 and 48 h.

The cell viability was estimated by MTT assay. MTT solution (Biotium, #30006) was heated to 37°C and then 10 µl were added to the each well. After 4 h incubation at 37°C in 5% CO_2, 200 µl DMSO was added to dissolve the formazan salts. The absorbance was measured at 570 nm with spectrophotometer (Versa Max, Molecular Device, Sunnyvale, USA).

TUNEL assay for apoptosis

DNA fragmentation was detected by labelling apoptotic cells with specific staining while using commercial *in situ* apoptosis detection kit (Apoptag Plus Peroxidase *In Situ* Apoptosis Detection Kit, S7101, Millipore, USA). All cultured cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) at 4°C for 30 minutes. After fixation, all cultured cells were washed twice with PBS and incubated with 3% H_2O_2 for 5 minutes at room temperature. In total, 75 µl of equilibrium buffer was added into the plates. Then, 55 µl of working TdT strength enzyme was added and the cells were incubated for 1 h at 37°C. After that, stop wash buffer was added, the cells were washed with PBS and 65 µl anti-digoxigenin peroxidase was added. The cells were washed by PBS and after incubated with 75 µl diaminobenzidine (DAB) for 5 minutes, the cells were re-washed with distilled water. The last step was counterstaining with Mayer's solution. The cells were washed with distilled water after 5 minutes of incubation.

Immunocytochemistry

Cultured Colo-320 and Colo-741 cells were assessed immunocytochemically for binding of antibodies against caspase-3, cytochrome-c and FasL. All cultured cells were fixed with 4% paraformaldehyde in PBS at 4°C for 30 minutes. Tween 20 (Sigma-Aldich) was added for permeabilization for 15 minutes. The cells were washed with PBS and endogenous peroxidase activity was quenched by incubation with 3% H_2O_2 for 5 minutes at room temperature. After washing cells with PBS three times for 5 minutes, primary antibodies caspase-3 (sc-7272, Santa Cruz Biotechnology, Inc., USA), cytochrome-c (sc-13156, Santa Cruz Biotechnology, Inc., USA) and FasL (sc-834 Santa Cruz Biotechnology, Inc., USA) were added and incubated overnight at 4°C. Biotinylated secondary antibody (Histostain-Plus, IHC Kit, HRP, 859043, Thermo Fischer) was added and incubated for 30 minutes followed by PBS wash (x3) for 5 minutes. Strepavidin-peroxidase complex (100 µl) was added to cultured cells. Cells were then washed by PBS and DAB was added and incubated for 5 minutes for enhancement of immuno-labelling. DAB was washed with distilled water. Cells were counterstained with Mayer's hematoxylien for 5 minutes and mounted with mounting medium (Merck Millipore, 107961, Germany). All specimens were examined using a light microscope (Olympus BX40, Tokyo, Japan).

Staining of caspase-3, cytochrome-c and FasL was also graded semi quantitatively using the H-SCORE that was calculated with the following equation: HSCORE=Σл (i+1), where i is the intensity of staining with a value of 1, 2 or 3 (mild, moderate, or strong, respectively) and л is the percentage of cells stained with each intensity, varying between 0 and 100%.

Analysis of the data

Results were expressed as mean ± standard deviation (SD). The results were analyzed using GraphPad Prism 7 software. Differences among groups were analyzed statistically with Kruskal-Wallis and Mann-Whitney where appropriate. A p value of <0.05 was considered as significant and a p value of <0.01 was considered as highly significant.

Results

Cell viability and cytotoxicity

Colo-320 and Colo-741 cells were treated with different concentrations of (5-100 µg/ml) DCM and aqueous phase extracts of *Corchorus olitorius* L. for 24 and 48 hours. Both extracts at 50 µg/ml concentration were more effective at inhibiting Colo-320 and Colo-741 cell growth when compared with other dilutions (Figure 1 and 2). DCM phase extract inhibited growth of Colo-320 and Colo-741 cells in a dose dependent manner (Figure 2).

Cell morphology

After treated with both DCM and aqueous phase of *Corchorus olitorius* L. extracts, the morphology of Colo-320 and Colo-741 cells were similar to control

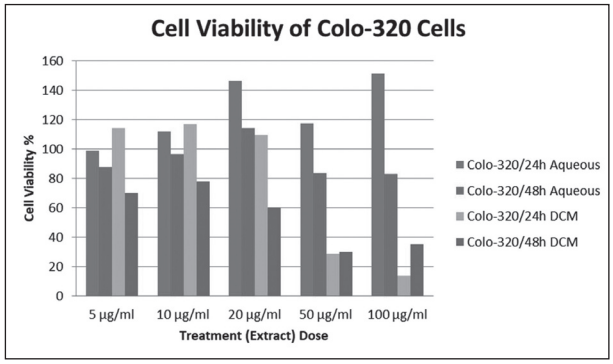

Figure 1. Effect of aqueous and DCM phase extracts of *Corchorus olitorius* L. on cell viability of Colo-320: Colo-320 cells were treated with different concentrations of aqueous and DCM phase extracts of *Corchorus olitorius* L. for 24 and 48 h. Negative control- no cells, positive control- non-treated cells.

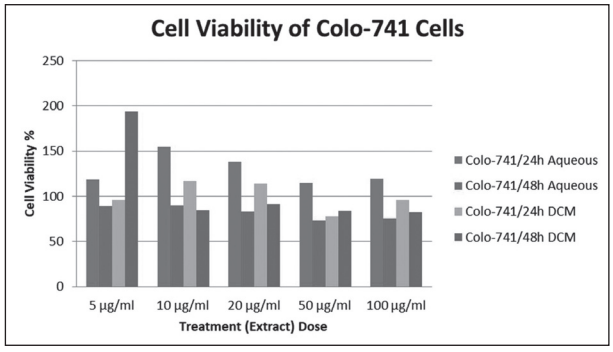

Figure 2. Effect of aqueous and DCM phase extracts of *Corchorus olitorius* L. on cell viability of Colo-741 cells: Colo-741 cells were treated with different concentrations of aqueous and DCM phase extracts of *Corchorus olitorius* L. for 24 and 48 h. Negative control- no cells, positive control- non-treated cells.

group (Figure 3 A-D). Larger vacuoles were observed in Colo-741 cells that were treated with both extracts (Figure 3 D, F) however no vacuole formation was detected in Colo-320 cells (Figure 3 C, E).

Apoptotic effects of Corchorus olitorius L. extracts in Colo-320 and Colo-741 cells

TUNEL assay was used in both cell lines that were incubated with 50 µg/ml concentration of extracts for 24 h. Unlike Colo 320 cells, the number of TUNEL positive cells that were treated with both extracts were highly significant in Colo-741 cells when compared with both control group ($p<0.01$, Table 1) and Colo-320 cell lines ($p<0.01$, Table 1) (Figure 4D-F).

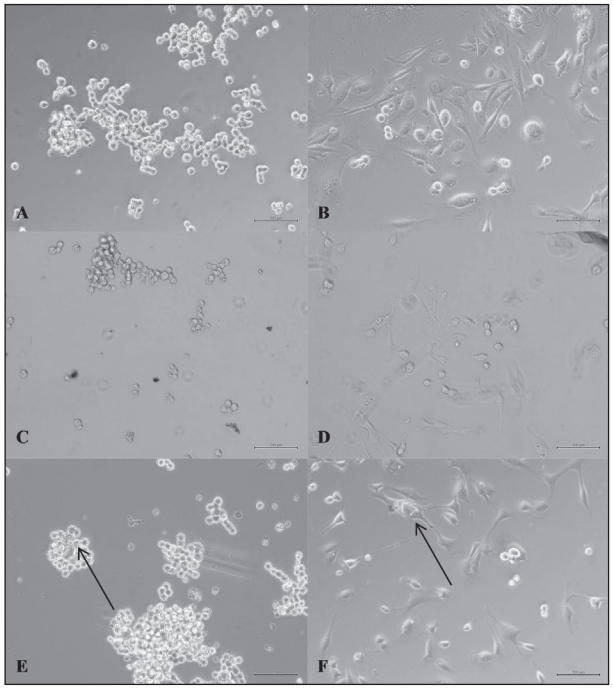

Figure 3. Colo-320 and Colo-741 cells imaged under the inverted microscope: (A) Colo-320 cells and (B) Colo-741 cells (C) DCM phase extract treated Colo-320 cells (D) DCM phase extract treated Colo-741 cells (E) Aqueous phase extract treated Colo-320 cells (F) Aqueous phase extract treated Colo-741 cells. Scale bars= 100 µm.

Immunocytochemical evaluation

Caspase-3 immunostaining was strong in Colo-320 cells treated with DCM phase extract and weak to moderate in aqueous phase treated Colo-320 cells (Table 2). The H-SCORE and immunoreactivity results were in parallel with immunostaining intensity, where H-SCORE for caspase-3 was significantly higher in Colo-320 cells treated with DCM phase extract ($p<0.05$, Table 3) (Figure 5A-C). On the other hand, H-SCORE for aqueous phase treated Colo-320 cells was not significant when compared with control group ($p>0.05$, Table 3). Immunostaining of caspase-3 in Colo-741 cells was moderate to strong for DCM phase and weak to moderate for aqueous phase (Table 2). As shown in Figure 5, immunoreactivity of caspase-3 in Colo-741 cells was higher in extract treated cells than control group. H-SCORE results revealed that, immunolabelling was significantly higher in both DCM and aqueous extracts in comparison with control group, respectively ($p<0.01$, $p<0.05$, Table 3) in Colo-741 cells.

Table 1. The percentage of TUNEL positive Colo-320 and Colo-741 cells treated with DCM and aqueous phase of *Corchorus olitorius* L. at 50 µg/ml concentration for 24h.

	Colo-320 cells	Colo-741 cells
DCM phase	5.94 ± 2.19	72.5 ± 25.28 [a,b]
Aqueous phase	11.38 ± 6.68	70.43 ± 9.72 [a,b]
Control	4.14 ± 4.25	4 ± 8.94

Data are expressed as means ± SD and were compared by Mann-Whitney.

[a] The data was significant when compared with control group (p<0.01)

[b] The data was significant when compared with Colo-320 cell lines (p<0.01)

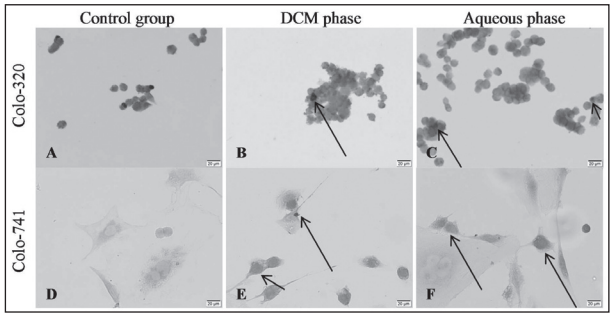

Figure 4. Apoptotic effects of *Corchorus olitorius* L. DCM and aqueous extracts in Colo-320 and Colo-741 cell lines: (A) Colo-320 control cells, (B) Colo-320 DCM phase extract treated cells, (C) Colo-320 aqueous phase extract treated cells, (D) Colo-741 control cells, (E) Colo-741 DCM phase extract treated cells, (F) Colo-741 aqueous phase extract treated cells. Scale bars= 20 µm.

Immunostaining intensity for cytochrome-c was moderate to strong for both extracts in Colo-320 cells (Table 2). As shown in Figure 6, cytochrome-c immunoreactivity was higher in both extracts treated Colo-320 cells than in control group. Aqueous phase treated Colo-320 cells had shown significant increase in H-SCORE than control group ($p<0.05$, Table 3). Although DCM phase treated cells showed raise in H-SCORE, the increase was not statistically significant ($p>0.05$, Table 3). Cytochrome-c immunostaining

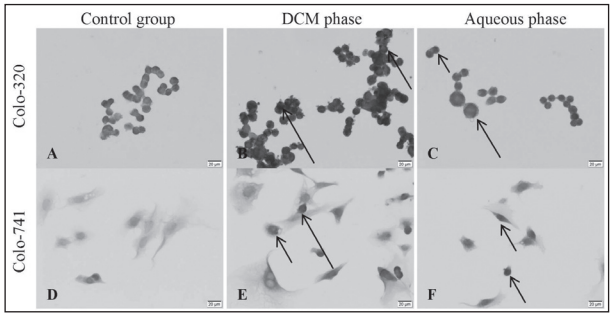

Figure 5. Apoptotic effects of *Corchorus olitorius* L. DCM and aqueous extracts in Colo-320 and Colo-741 cell lines: (A) Colo-320 control cells, (B) Colo-320 DCM phase extract treated cells, (C) Colo-320 aqueous phase extract treated cells, (D) Colo-741 control cells, (E) Colo-741 DCM phase extract treated cells, (F) Colo-741 aqueous phase extract treated cells. Scale bars= 20 µm.

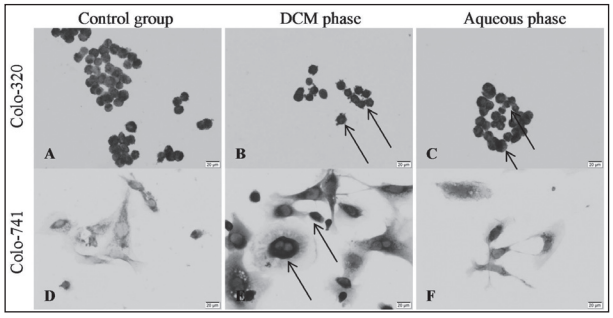

Figure 6. Immunoreactivity of cytochrome-c in Colo-320 and Colo-741 treated with 50 µg/ml DCM and aqueous extracts for 24h: (A) Colo-320 control cells (B) Colo-320 DCM phase extract treated cells (C) Colo-320 aqueous phase extract treated cells (D) Colo-741 control cells (E) Colo-741 DCM phase extract treated cells (F) Colo-741 aqueous phase extract treated cells. Scale Bars=20µm

was strong for DCM phase and moderate for aqueous extract treated Colo-741 cells (Table 2). DCM phase extract had shown increased immunoreactivity than aqueous phase and control group in Colo-741 cells, (Figure 6 D-F). According to H-SCORE of Colo-

Table 2. The intensity of caspase-3, cytochrome-c and FasL immunolabelling in Colo-320 and Colo-741 cells treated with DCM and aqueous phase of *Corchorus olitorius* L. at 50 µg/ml concentration for 24h.

	Colo-320 cells			Colo-741 cells		
	Control group	**DCM phase group**	**Aqueous phase group**	**Control group**	**DCM phase group**	**Aqueous phase group**
Caspase-3	+	+++	+/++	-/+	++/+++	+/++
Cytochrome-c	++	++/+++	++/+++	-/+	+++	++
FasL	+	+/++	++/+++	+/++	++/+++	++/+++

741 cells, both extracts had shown highly significant increase in immunolabelling than control group ($p<0.01$, Table 3).

FasL immunostaining intensity results showed that DCM phase was exerted weak to moderate effect whereas aqueous phase effect was moderate to strong in Colo-320 cells (Table 2) with similar patterns observed in immunoreactivity (Figure 7A-C). In addition, there was a decrease in H-SCORE in DCM treated Colo-320 cells (Table 3). Contrary to that, aqueous phase extract treated cells had shown significant increase in H-SCORE when compared with control group ($p<0.05$, Table 3). FasL immunostaining intensity was moderate to strong for both extracts in Colo-741 cells (Table 2). In addition, immunoreactivity was higher in both extracts in Colo-741 cells than control group (Figure 7 D-F). Both extracts showed significant increase in FasL H-SCORE while DCM phase being significantly higher than aqueous phase extract treated Colo-741 cells ($p<0.05$, Table 3). In addition, FasL immunolabelling highly significant in aqueous phase extract treated Colo-320 cells than Colo-741 cells ($p<0.01$).

Discussion

Corchorus olitorius L. is known to have medicinal properties in Mediterranean and Asian culture due to its rich phytochemical content (4,5,22). The plant had shown to have anti-inflammatory, antioxidant, antitumor and anticancer activities (2,8,14,22). According to a study by Li et al. (2012), *Corchorus olitorius* L. leaf extract at 12.5 μg/ml concentration triggered apoptosis by increasing caspase-9 activity as well as cytochrome c leakage from mitochondria which showed that the extract induced apoptosis via intrinsic pathway in HepG2, hepatocellular carcinoma cells (14). Additionally, another study reported that *Corchorus olitorius* L. leaf extract at 150 μg/ml concentration had caused cytotoxic effects on human multiple myeloma cells (ARH-77) and stated that these cytotoxic effects increased in dose-dependent manner (2). In our study, 50 μg/ml concentrations of both extracts were further investigated as other concentrations of extracts might exert either cytotoxic effects to the normal cells or not be able to induce apoptosis in cancer cells. Both studies

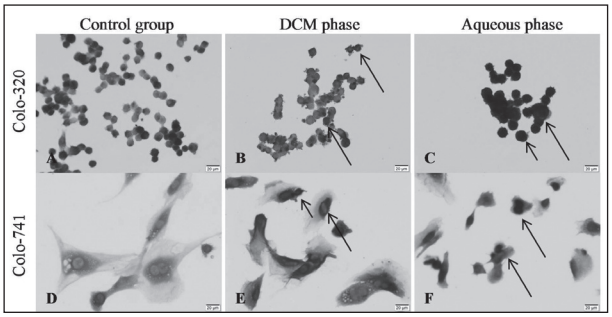

Figure 7. Immunoreactivity of FasL in Colo-320 and Colo-741 treated with 50 μg/ml DCM and aqueous extracts for 24h: (A) Colo-320 control cells, (B) Colo-320 DCM phase extract treated cells, (C) Colo-320 aqueous phase extract treated cells, (D) Colo-741 control cells, (E) Colo-741 DCM phase extract treated cells, (F) Colo-741 aqueous phase extract treated cells. Scale Bars=20μm

Table 3. The H-SCORE of caspase-3, cytochrome-c and FasL immunolabelling in Colo-320 and Colo-741 cells treated with DCM and aqueous phase of *Corchorus olitorius* L. at 50 μg/ml concentration for 24h.

	Colo-320 cells			**Colo-741 cells**		
	Control group	**DCM phase group**	**Aqueous phase group**	**Control group**	**DCM phase group**	**Aqueous phase group**
Caspase-3	258.8 ± 41.23	355.3 ± 19.69[a]	312.3 ± 41.99	218.3 ± 52.86	350.3 ± 18.94[a]	320.3 ± 52.45[a]
Cytochrome-c	317.7 ± 36.17	370.2 ± 30.29	366 ± 22.4[a]	208.9 ± 19.87	371.3 ± 11.83[a]	354.2 ± 22.5[a]
FasL	372.5 ± 19.18	300.2 ± 52.43	397.1 ± 6.57[a,b]	278.3 ± 45.49	382.3 ± 2.12[a,c]	360.7 ± 16.77[a,b]

Data is expressed as means ± SD and were compared by Mann-Whitney.
a The data was significant when compared with control group ($p<0.05$)
b The data was significant when compared with different cell lines ($p<0.01$)
c The data was significant when compared with Aqueous phase group ($p<0.05$)

had only used ethanol extract unlike our study where two different extracts were used at the same time on different cell lines (2,14). Different extracts might have different phytochemical contents which might explain the amount of concentrations used as treatment might be different than other studies. The MTT results stated that 24 and 48 h incubation showed similarities for cytotoxicity therefore further immunocytochemical experiments were continued with 24 h incubation period.

TUNEL assay results showed that DCM extract of *Corchorus olitorius* L. was nearly 12-fold more effective in Colo-741 than Colo-320 cell lines. The same pattern was observed in aqueous phase where the extract was more effective in Colo-741 cell lines. Therefore, the extracts are found to be more effective in metastatic than primary cells in terms of apoptotic DNA fragmentation.

There are number of proteins and two main pathways, which play role in the process of apoptosis. Presence of some proteins in apoptosis such as FasL and caspase-8 show that apoptosis is triggered by extrinsic pathway. On the other hand, cytochrome-c, caspase-9 and Apaf-1 are indicators of intrinsic pathway (19). Our results showed that immunoreactivity of cytochrome-c was significantly higher in both Colo-320 and Colo-741 cells incubated with aqueous phase extract than control group. However, DCM phase extract had shown to significantly increase immunoreactivity of cytochrome-c in only Colo-741 cells. The results indicate that both extracts were effective in triggering intrinsic pathway in Colo-741 cells however; only aqueous phase extract was able to induce apoptosis via intrinsic pathway in Colo-320 cells.

The immunoreactivity of FasL had shown a decrease in Colo-320 cells which were incubated with DCM phase extract when compared with control group. This might indicate that, DCM phase extract did not induce apoptosis through extrinsic pathway in Colo-320 cells. Contrary to Colo-320 cells, FasL immunoreactivity was significantly higher in Colo-741 cells treated with DCM phase. Therefore, these results suggest that DCM phase extract had shown to induce apoptosis in only Colo-741 cells through extrinsic pathway. In both cell lines, aqueous phase was able to significantly increase immunoreactivity of FasL being more effective in Colo-320 cells. This might indicate that aqueous phase extract might be a mediator to induce apoptosis via extrinsic pathway.

Caspase-3 immunoreactivity was significantly higher in DCM phase extract treated Colo-320 cells indicating that downstream of apoptotic pathway had been reached. On the other hand, immunoreactivity of cytochrome-c and FasL were not significant for DCM phase extract treated Colo-320 cells. This result might suggest that, apoptosis might be triggered with the help of other proteins which are found in the flow of apoptosis through different mechanisms that need further evaluation. Aqueous phase extract did not significantly increase in caspase-3 immunoreactivity in Colo-320 cells while the immunoreactivity of cytochrome-c and FasL in Colo-320 cells were significantly higher than control group. It should be stated that the cells were incubated with extracts for 24 h and incubation time might not be enough to trigger significant raise in caspase-3 immunoreactivity. Additionally, in Colo-741 cells, both extracts significantly increased caspase-3 immunoreactivity indicating that, apoptosis was triggered in metastatic colon adenocarcinoma cell lines.

DCM extract content is rich in carotenoids, α-tocopherols and polyunsaturated fatty acids (9,23). Carotenoids and omega-3 fatty acids had shown to suppress Nuclear Factor-κB (NFκB) activation and Bcl-2 expression and induced apoptosis in cancer cells (12,24). In addition, α-tocopherol is a lipophilic compound which can only be dissolved in organic solvents such as DCM. Therefore, α-tocopherol is expected to be more effective in DCM phase extract. However, it was stated that gamma and delta forms of tocopherol are rather more effective than alpha form in terms of inhibiting colon cancer cell growth and induction of apoptosis (25,26). The difference in the apoptotic effect of DCM phase might be due to different reactions of different cancer cell lines to extract and its phytochemical content.

The other extract that was used in the study was aqueous, which contains natural anticancer compounds such as quercetin and caffeoylquinic acid (6–8). Quercetin contains hydroxyl groups which act as electron acceptor thus has antioxidant properties. Its cancer preventive effects might be due to its reactive oxygen species (ROS) scavenging ability as well

as induction of apoptosis in cancer cells by activating Bax and Bak proteins and increase in p53 expression (27,28). A study by Handoussa *et al.* (2013) stated that *Corchorus olitorius* L. extract significantly increased glutathione (GSH) and superoxide dismutase (SOD) activities in carrageenan-induced inflammation in rats (8). Quercetin had shown to induce apoptosis by increasing intracellular ROS in colon cancer (28). In addition, a derivative of quercetin caused cell death by inhibiting Bcl-2 and increasing Bax expression via intrinsic pathway in HCT-116 human metastatic colon cancer cells (29).

The other constituent of aqueous phase extract, caffeoylquinic acid, is a sub-group of chlorogenic acid, is known to be an antioxidant polyphenolic compound which is predominantly found in coffee (30). It is also regarded as one of the most abundant phytochemicals in *Corchorus olitorius* L. (7,8). Chlorogenic acid had shown cytotoxic effects in HT-29 colon cancer cells by increasing caspase-3 and decreasing Bcl-2 activity. It was also stated that chlorogenic acid suppressed heat shock protein 70 and therefore decreased tumour growth (30). In addition, 5-caffeoylquinic acid showed decrease in cell viability and induced apoptosis in colon adenocarcinoma cells (31).

All of these studies suggest that phytochemicals that are found in aqueous phase extract is effective in induction of apoptosis in different colon cancer cell lines. Although aqueous phase extract had activated both intrinsic and extrinsic pathways it was not able to initiate caspase-3 activity in primary colon cancer cells. On the other hand, aqueous phase extract did activate all three apoptotic pathway proteins in metastatic colon cancer cells which triggered both intrinsic and extrinsic signalling pathways.

Induction of apoptosis in cancer cells has been a part of cancer treatment throughout the years (18). However, apoptotic pathway that triggers cell death might show difference in different cancer cells. For instance, *Corchorus olitorius* L. extract induced apoptosis through intrinsic pathway in hepatocellular carcinoma cells (14) however our results suggested that specifically aqueous phase extract triggered both intrinsic and extrinsic apoptotic pathways in both cell lines. Overall, both extracts were more effective in Colo-741, metastatic colon adenocarcinoma cell lines. The fact that apoptotic cells are higher in metastatic colorectal carcinomas may be explained by metastatic cells being under more cellular stress as they are expected to accumulate mutations more than the primary tumours (32). When coupled with phytochemicals in *Corchorus olitorius* L. extract, apoptosis might be triggered in a more readily manner in the metastatic cells that are already in a greater cellular stress as opposed to primary colorectal adenocarcinomas (32).

Conclusion

Cancer incidence and mortality is quite high and phytomedicinal approach to the treatment is increasing as the treatment is less invasive. In our study, *in vitro* proapoptotic activity of DCM and aqueous extracts of *Corchorus olitorius* L. were investigated for the first time in colon adenocarcinoma cells. Two different extracts of *Corchorus olitorius* L. leaves had shown to induce apoptosis in both cell lines at 50 μg/ml concentration. In conclusion, extracts induced apoptosis in both cancer cell lines while being more effective in metastatic colon adenocarcinoma cell lines. This study indicates that extracts might have potential anticancer effects and possibility to be used as precursor to phytomedicinal colon cancer treatment. This study will be a guide to future studies where the apoptotic effects of *Corchorus olitorius* L. extracts might further be investigated with different cancer cell lines and *in vivo* studies.

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