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Apoptotic Effects of *Opuntia ficus indica* L. Seed Oils on Colon Adenocarcinoma Cell Lines †

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Abstract: *Opuntia ficus indica* L. fruit (cactus pear) seed oil is used in traditional and complementary therapies for its numerous health benefits. The aim of this study was to analyse of the fatty acid content and apoptotic induction effects of spiny and thornless *Opuntia ficus indica* L. seed (CPS) oils. Spiny and thornless *Opuntia ficus indica* L. seed oils obtained by supercritical CO_2 extraction method and analyzed by GC-MS. Different concentrations of almond oils were incubated for 24 h and 48 h with Colo-320 and Colo-741 cells. Cell growth and cytotoxicity were measured by MTT assays. TUNEL assay was used to detect DNA fragmentation in both cell lines. Linoleic acid was dominant fatty acid followed by oleic acid, palmitic acid and elaidic acid in both type of seed oil. In MTT, spiny CPS oil was found to be active against Colo-320 and Colo-741 cells with 1:16 dilution for 48 h. Also, 1:8 and 1:16 dilutions of thornless CPS oil showed significant reduction in the number of viable cells in Colo-320 and Colo-741 cells, respectively. The number of TUNEL positive cells were significantly higher in Colo-320 cells treated with thornless CPS when compare with control group ($p < 0.05$).We conclude that thornless CPS oil may have anticancer effect on primer colon adenocarcinoma cell lines. The effect can be explained by inducing apoptosis. Thus, they could be a potential novel therapeutic agent in colon cancer therapy.

Keywords: *Opuntia ficus indica*; seed oil; anticancer activity

1. Introduction

Opuntia ficus indica (L.) is a member of Opuntiodea subfamily among the Cactaceae family. *Opuntia ficus indica* (L.) fruit (cactus pear) is popular fruit throughout the Mediterranean area and contains minerals, vitamins, fatty acids, sugar, polyphenols and flavonoids. Many studies showed that cactus pear can reduce oxidative stress and may prevent cancer, inflammation and ulcers [1]. The chemical analysis of cactus pear pulp, skin and seeds were studied. Especially, cactus pear seed (CPS) oil is rich from linoleic acid, oleic acid, palmitic acid and fatty acids. Especially, animal studies have provided evidence that CPS oil is closely associated with reducing blood glucose levels and improving lipid profile [2]. Spiny (wild) and thornless (culturvated) form of *Opuntia ficus indica* L. grow in Cyprus. No data has been mentioned about seeds fatty acids composition effects of spiny

(wild) and thornless (cultivated) form of Cyprus *O. ficus indica* L. Also, there are no reports addressing the effect of CPS oil on colon cancer with in vitro and in vivo studies.This study determines the fatty acid composition and in vitro anticancer activity of spiny and thornless form of Cyprus CPS oils from Northern Cyprus (NC) in primary (Colo-320) and metastatic (Colo-741) colon carcinoma cell lines. In addition, we aimed to determine the molecular apoptotic effects of both seed oils in both cell lines.

2. Experimental Procedure

Spiny and thornless cactus pear mature fruits were collected in summer from İskele province of Northern Cyprus. Fruits pulps were removed from seeds. The seeds were washed and dries in atmospheric conditions. CPS oils were obtained by using supercritical CO_2 extraction (Super critical fluid extraction 100-2-FMC system) (Thar Instruments Inc.) method. Fatty acid composition of spiny and thornless CPS oils were investigated by gas chromatography–mass spectrometry (GC-MS) analysis [3]. Colo-320 (ATCC catalogue: CCL 220) and Colo-741 cell lines (ECACC 93052621) are maintained in culture in RPMI-1640 medium (Biochrom, FG1215, Berlin, Germany), 10% FBS (Capricorn Scientific, FBS-11B, Ebsdorfergrund, Germany), 1% penicillin-streptomycin (Biochrom, A2213, Berlin, Germany) and 1% glutamine (EMD Millipore, K0282, Darmstadt, Germany) in a humidified atmosphere at 37 °C and 5% CO_2. The cell viability estimation was done by MTT assay, reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan salts. 10 µL MTT solution (5 mg/mL MTT in PBS) was added to the each well and incubated for 4 h at 37 °C. Then 50 µL DMSO was added to dissolve the formazan salts. The absorbance was measured at 540 nm with spectrophotometer (Versa Max, Molecular Device, Sunnyvale, CA, USA). 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, Burlington, MA, USA). 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 where appropriate. A p value of <0.05 was considered as significant.

3. Results and Discussion

The fixed oil yield of seeds and fatty acid composition of spiny and thornless form of *Opuntia ficus indica* from Northern Cyprus were investigated for the first time in this study. Compounds of spiny CPS oil were linoleic acid (55,9%), oleic acid (17.6%), palmitic acid (12.4%) and elaidic acid (4%). Thornless CPS oil contains linoleic acid (60.1%), oleic acid (15.6%), palmitic acid (12.3%) and elaidic acid (4.1%). According to the results of studies, linoleic acid is major fatty acid ranging from 50 to 60% in different origin spiny and thornless CPS oils [4–6]. Among the two spices CPS oil from Cyprus, thornless type CPS oil contained linoleic acid (omega-6) more than an amount of spiny CPS oil.

Colo-320 and Colo-741 cells were treated with spiny and thornless CPS oils at various dilutions for 24 and 48 h. CPS oils inhibited the growth of Colo-320 and Colo-741 cells in a dose-and time-dependent manner. Our results showed that 1:16 dilution spiny CPS oil was more effective in inhibiting Colo-320 and Colo 741 cell growth when compared with other dilutions. On the other hand, 1:8 and 1:16 dilutions of thornless CPS oil showed significant reduction in the number of viable cells in Colo 320 and Colo-741 cells, respectively (Figure 1).

Colo-320 cells are refractile, semi adhesive and rounded cells in standard culture condition. Colo-741 cells are fibroblast-like cells that grow with typical fibroblast colony morphology after 24 h in culture. Number of Colo-320 cells decreased after treated with spiny and thornless CPS oils. Apoptosis is an important process of programmed cell death which plays a key role in maintaining cellular homeostasis [7]. TUNEL assay was used to determine the apoptotic effects of CPS oils in Colo-320 and Colo-741 cell lines that were incubated for 48 h. The number of TUNEL positive cells that were treated with both oils were highly significant in Colo-320 cells when compared with both control groups ($p < 0.05$, Table 1) (Figure 2A–C). TUNEL positive cells number significantly higher in Colo-320 cells treated with thornless CPS when compare with control group ($p < 0.05$). On the other hand, the number of TUNEL positive cells were not significant in Colo-741 cells treated with both CPS oils when compared with control group ($p > 0.05$, Table 1) (Figure 2D–F). The results showed

that thornless CPS oil was more effective in primary, Colo-320 cell lines than control group. Several in vitro studies have shown that linoleic acid has growth-inhibitory and pro-apoptotic effects on different type of cancer cell lines [8]. For this reason, the inhibitory effect of thornless CPS oil is due to the high linoleic acid content which is a known compound with an anticancer effect in cancer cells.

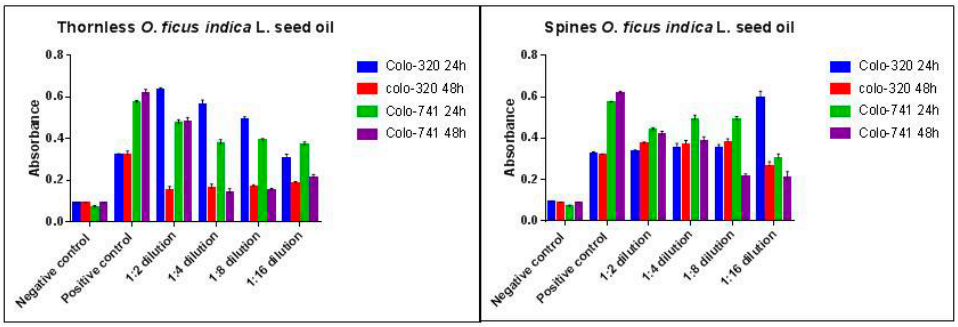

Figure 1. Effect of spiny and thornless cactus pear seed oils on cell viability of Colo-320 and Colo-741 cells. Colo-320 and Colo-741 cells were treated with different dilutions of seed oils for 24 and 48 h. Viability was quantitated by MTT assay. The values are expressed as mean ± S.D.

Table 1. The percentage of TUNEL positive Colo-320 and Colo-741 cells treated with spiny and thornless CPS oils for 48 h.

	Control Group	Thornless CPS Oil	Spiny CPS Oil
Colo-320 cells	3.91 ± 0.72	50.66 ± 34.59 *	40 ± 37
Colo-741 cells	3.38 ± 3.22	9.53 ± 2.34	11.03 ± 9.6

Data are expressed as means ± SD and were compared by Kruskal-Wallis. * The data was significant when compared with control group ($p < 0.05$).

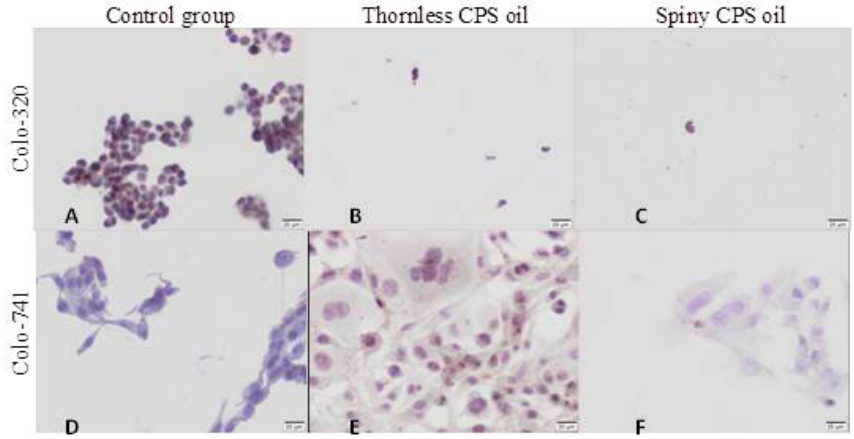

Figure 2. Apoptotic effects of spiny and thornless CPS oils in Colo-320 and Colo-741 cell lines. Apoptosis was determined by TUNEL assay. (**A**) Colo-320 control cells, (**B**) Colo-320 thornless CPS oil treated cells, (**C**) Colo-320 spiny CPS oil treated cells, (**D**) Colo-741 control cells, (**E**) Colo-741 thornless CPS oil treated cells, (**F**) Colo-741 spiny CPS oil treated cells. Scale bars = 20 μm.

4. Conclusions

In conclusion, the results of the present study showed that spiny and thornless *Opuntia ficus indica* seed oils rich in linoleic acid mediate anticancer effect with colon carcinoma cells. These in vitro results are needed to be evaluated with in vivo studies to for possible use of *Opuntia ficus indica* seed oil in the prevention and management of cancer.

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Conflicts of Interest: The authors declare no conflict of interest.

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