European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 22-01-2019; Accepted: 25-02-2019
www.biosciencejournals.com
Volume 7; Issue 2; March 2019; Page No. 62-65

The protectıve effect of shark cartilage (SC) and lıver oıl (SLO) against to colon and lung cancer by cell culture

Elvan Akgül[1], Nadir Koçak[2], Tuğçe Duran[3], Vasfiye Betül Uçar[4], Mine Dosay Akbulut[5]
[1] PhD and Nurse in Afyon Kocatepe Uni. Medical Faculty Afyon, Turkey
[2] Asist. Profesore Dr. Selçuk Uni. Medical Faculty, Medical Genetic Dep. Konya, Turkey
[3, 4] Master Student. Selçuk Uni. Medical Faculty, Medical Genetic Dep. Konya, Turkey
[5] Associate Profesore, Dr. Afyon Kocatepe Uni. Veterinary Faculty, Medical Biology and Genetics Dep, Afyon, Turkey

Abstract
It is a fact that cancer disease is one of the biggest health problems today. It is important for cancer patients to maintain their immune system strong. Many research have been carried out to find better cancer treatments. The findings indicate that; the medicinal plants are widely used for the treatment of diseases as an complementary medicine. In treatment of this illness, additional to standard methods, sharks, with their cartilage and liver oil, has been presented as a one of these new sources in this study. Cell culture is a preferred method in terms of reducing the use of experimental animals, in that it can be done much more easily than many studies with experimental animals. With this study; it was aimed to determine the protective effect of SC (shark cartilage-dogfish cartilage) and SLO (shark liver oil-dog liver oil) as therapeutic agents against to colon cancer and lung cancer lines prepared by cell culture.
For lung cancer; The H209 cells from the alum were cultured and "CaCo-2 / An1 (Human Colon Adenocarcinoma-human)" was used as a colon cancer line in our study.
In comparison of SC and SLO effectiveness against to lung and colon cancer lines; SC application was more effective showing its killing cancer cells effect in shorter time with lower doses compare to SLO application in colon cancer lines; the SLO was a little more effective, showing its killing effect in lower doses with the same period of time; compare to SC application in lung cancer lines.

Keywords: colon and lung cancer, shark cartilage and liver oil, cell culture, and cancer line

1. Introduction
Cancer determined as malignant cell growth as a result of abnormal and uncontrolled cell division. It is a fact that cancer is one of the biggest health problems in these days. The highest seen cancer types for males and females in the world are; stomach, lung, breast, colon-rectum and cervical cancers. The incidence can change on the bases of the; sex, age, organs developed by the cancer and environmental factors [1].
The WHO published reports indicated that the medicinal plants are widely used for the treatment of diseases with the name of complementary medicine, and the interest to these plants is increasing every day. Those who are interested in alternative treatment methods from cancer patients use many plants, primarily the stinging nettle, and some other herbals like ginko, ginseng, echinacea etc. to support or relax medical treatment.

1.1. Shark Cartilage Chemical Structure (SC)
Shark cartilage is a very potent inhibitor of tumor formation. The protein content of the shark cartilage is similar to that of bovine cartilage inhibiting tumor formation. In the shark, nearly 10 times the amount of cartilage in the structure of the cattle is found. Shark cartilage is 1000 times stronger than the cattle cartilage and it contains 100,000 times more tumor suppressor materials with structural comparison[2]. In 1988, Patricia D'Amore stated that metaztase was due to the development of new blood vessels, and it was stated that the prevention of vascularization may be the causative agent in preventing metaztases. It has been indicated that inhibition of a single vascular system may cause death or inhibition of many tumor cells [3].
Some important specific molecules have been found in cartilage. These are chondromodulin-1, thrombospondin-1, type XVIII-derived endostatin, SPARC (acidic and cysteine rich secretory protein) and type II collagenous derivative N-terminal propeptide (PIIBNP). These molecules have been tested for their anti-tumor or antiangiogenic effects in different studies [4].

1.2 Shark Liver Oil (SLO) and Its Protective Effect
Alkylglycerols and squalene have an important role in fight against to infections and cancer. Shark liver oil includes a large amount of alkylglycerol, squalene and n-3 EFA. Therefore, it can be used in the treatment of cancer, especially in the treatment of body resistance in radiotherapy and in the treatment of infectious diseases [5].
Fish oils are known to contain different active compounds related to different functions of body and regulation of cell activity. High level of 1-O-alkylglycerols has been found in shark liver oils with ability of enhancing the immune system. In a study, it was determined that 1-O-alkylglycerols derived from fish oils were effective in the antitumor content and combined treatment of different types of cancer [6].
With this study, we aimed to determine the protective effect

of SC (shark cartilage) and SLO (shark liver oil) as therapeutic agents in colon cancer and lung cancer lines prepared by cell culture on the genetic bases.

1.3 Cell Lines
The cell lines first made significant progress in scientific research by the cultivation of HeLa cell lines in 1951. Today cell cultures are widely used in cancer researches and in the field of virology.

1.4 Cell Culture
Growing and propagation of pieces, taken from the living tissue or organ, in invitro medium is called cell culture. Cell culture is a preferred method in terms of reducing the use of experimental animals, in that it can be done much more easily than many studies with experimental animals[7,8].
The aim of cell culture is to keep a group of cells alive, to multiply for further study, to keep them frozen for use when necessary. To increase the number of experiments that can be done to increase the number of cells, the experiment can be repeated with the cells in the same passages, which need to be kept of these passages of the cells in frozen. At each stage, the viability of the cells has a great importance. Providing sterile working conditions is a major factor in success of cell culture[9].

2. Materials and Method
For lung cancer; The H209 cells taken from the Şap Institute were cultured in fresh medium containing 10% FBS (Fetal Bovine Serum), 1% Antibiotic (Penicillin / streptomycin) and RPMI1640 medium in a oven with a 5% CO2 at 37 ° C.
"CaCo-2 / An1 (Human Colon Adenocarcinoma-human)" was used as a colon cancer line in our study. Caco-2 cells taken from the Şap institute were cultured in fresh medium containing 10% FBS (Fetal Bovine Serum), 1% Antibiotic (Penicillin / streptomycin) and DMEM medium in a oven with a 5% CO2 at 37 ° C.
When the cells covered 80-90% of the T25 flask surface, they were treated with 2 ml trypsin-EDTA. Approximately 4-5 minutes later, cells were developed and 4 ml of fresh medium containing 10% FBS (Fetal Bovine Serum), 1% Antibiotic (Penicillin / streptomycin) and RPMI 1640 medium was added to stop trypsinization. Then, the cell suspension was taken into a 15 ml volumetric tube and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded and fresh medium containing 10% FBS (Fetal Bovine Serum), 1% Antibiotic (Penicillin / streptomycin) and RPMI1640 medium was added to the pellet and pipetted thoroughly. After the pipetting, cells were sown to flask for passage. Cells were passaged according to cell density and proliferation.

2.1. MTT analysis
Cells harvested with trypsin-EDTA were distributed in 96 wells to obtaine as at least 3000 cells. Cells incubated at 37 ° C with 5% CO2 for 24 h were added to 96 wells, then the medium was discarded. 100 µl of fresh medium prepared with shark liver oil and shark cartilage prepared on doses of 40 mg / l, 80 mg / l, 120 mg / l, 160 mg / l and 200 mg / l were added. Before the measurement at appropriate times, the applied medium was removed. On top of this, 50 µl of 5 mg / ml MTT solution was added. After incubation for 3 hours at 37 ° C with 5% CO2, the MTT solution was discarded. DMSO was then added to the cells in the dark place. Measurements were taken at 570 nm wavelength on an ElisaReader (BIOTEK) instrument [10].

3. Results
MTT Analysis results for lung cancer; For SC H209;

Table 1

control	0,559333
80 mg/l	0,436083
120mg/l	0,245667
160 mg/l	0,371583
200 mg/l	0,513708

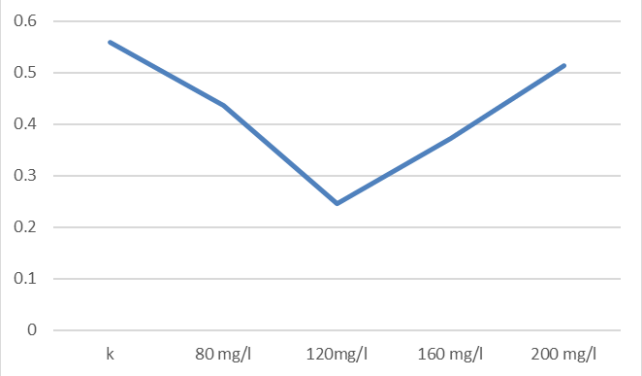

Fig 1: IC50 effective dose-value graph (48 hours and 120 mg dose).

Table 1: For SLO H209;

control	0,602667
80 mg/l	0,246167
120mg/l	0,455833
160 mg/l	0,467958
200 mg/l	0,505042

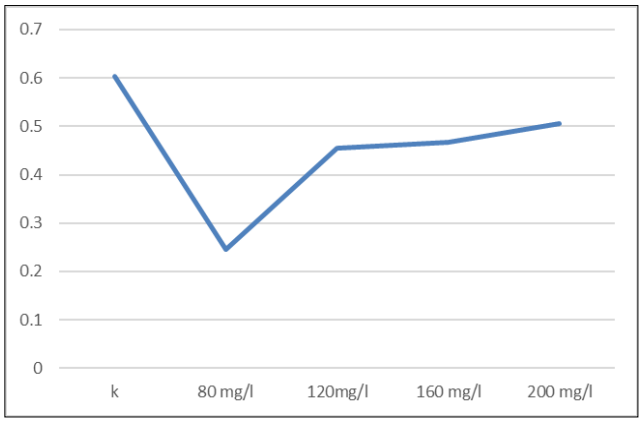

Fig 2: IC50 effective dose-value graph (48 hours and 80 mg dose).

Table 3: Cell culture results for colon cancer; For SC Caco-2;

control	0,5495
80 mg/l	0,504958
120mg/l	0,338333
160 mg/l	0,369444
200 mg/l	0,444

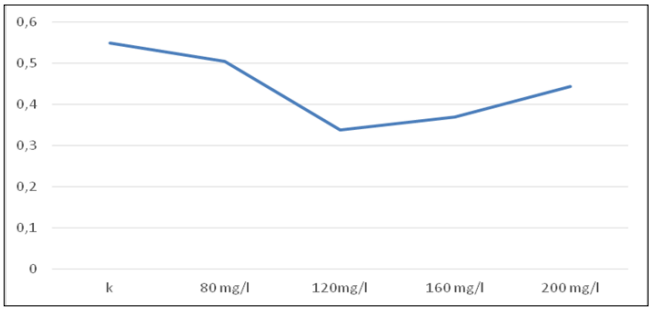

Fig 3: IC50 effective dose-value graph (6 hours and 120mg dose).

Table 4: SLO for Caco-2;

control	0,633
80 mg/l	0,420167
120mg/l	0,416292
160 mg/l	0,365417
200 mg/l	0,364583

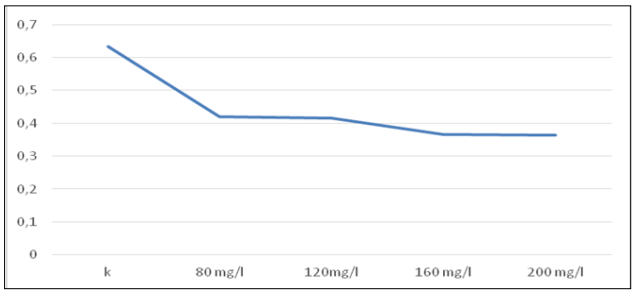

Fig 4: IC50 effective dose-value graph (at 24 hours and 200mg dose).

The findings of SLO and SC, lung and colon cancer used in the treatment are evaluated as having recovered by causing damage and tumor cells to regress.

In cell culture study using Caco-2 colon cancer cell line and SC; The IC50 effective dose value was found to be 120 mg / l at 6 hours. This means that SC can most effectively kill cancer cells at 6 hours and 120mg doses.

In cell culture study using Caco-2 colon cancer cell line and SLO; The IC50 effective dose value was found to be 200 mg / l at 24 hours. This means that SLO can most effectively kill cancer cells at 24 hours and 200mg doses.

H209 In cell culture study using lung cancer cell line and SC; The IC50 effective dose was found to be 120 mg / l per 48 hours. This means that SC can most effectively kill cancer cells at 48 hours and 120mg doses.

H209 In cell culture study using lung cancer cell line and SLO; The IC50 effective dose was found to be 80 mg / l at 48 hours. This means SLO can most effectively kill cancer cells at 48 hours and 80 mg dosing.

In comparison of SC and SLO effectiveness against to lung and colon cancer lines; SC application was more effective showing its killing cancer cells effect in shorter time with lower doses compare to SLO application in colon cancer lines; the SLO was a little more effective, showing its killing effect in lower doses with the same period of time; compare to SC application in lung cancer lines.

4. Discussion

Apelin is the endogenous ligand for the G protein-bound orfan apelin receptor (APJ). Distribution of apeline in the hypothalamus, especially in the hypothalamic areas such as arcuate, supraoptic and paraventricular nuclei suggests that the presence of APJ in the testis, prostate and breast tissues may have important physiological effects on the reproductive system. In one study, concentrations of 0.1, 1 and 10 nM of apelin-13, 1, 10, 100 nM of estrogen hormone were applied to the estrogen sensitive human breast cancer cell line (MCF-7). Doses of apelinin, 1 and 10 nM and doses of 1, 10, 100 nM of estrogen hormone were simultaneously cultured and incubated for 24 hours. The effects of these substances on MCF-7 cell viability were determined by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay. The results were determined as% cell viability value. Apelin-13 applied to MCF-7 cell line decreased cell viability ($p < 0.05$) and estrogen increased cell viability ($p > 0.05$). Apelin-13 reduces the viability of MCF-7 cell lines (including in estrogen-induced groups), suggesting that the proinflammatory effect is estrogen receptor-induced and apelin-13 may be used as an anti-carcinogenic agent. In this study apelin-13, which is used in cell culture as anti-cancer, is similar to the healing effects of SC and SLO used in our study [11].

A study showing that Cetraria aculeata liquefied extract has a weak cytotoxic effect on cancer cells of HeLa (human cervical adenocarcinoma), 5RP7 (cancerous rat embryo fibroblast cell line) and A549 (cancerous human alveolar basal epithelial cell line) cancer cells [12].

Urginea maritima (Um) is a plant specific to the Mediterranean region. In a study conducted, it was investigated whether the cytotoxic effect of the Um explant on cancer cells. A variety of extracts were prepared and these extracts were administered alone or in combination with Gemcitabine and / or Cisplatin to A549 Small Cell Lung Carcinoma (NSCLC) cell cultures. As a result, Umbilical Extract was observed to be more cytotoxic than Cisplatin, Gemcitabine and Umium leaf extract. In this study, it was recorded for the first time in the literature that the umbilical cord may be a drug candidate for the treatment of solid tumors. The cytotoxic effect on cancer cells in this study was similar to the effects of SC and SLO in our study with extirpation of ex- pression [13].

In another study; it was aimed to investigate the cytotoxic and apoptotic effects of cisplatin with different doses, applied to colon cancer cells (HT29) for 72 h. In the result of this study; cisplatin had a cytotoxic effect with a dose dependent situation for a 72 h applying period and they found that the optimum apoptotic dose was 50µM and the cisplatin induced apoptosis effectively using the caspase cascade signal pathway similar to our findings with SC and SLO [14].

In different study; ginger's (Zingiber officinale Roscoe) and its extract and 6-gingerol were tested for their inhibition effect on colon cancer biology--cancer cell proliferation and their angiogenic possibility on endothelial cell tubule formation. Antiproliferation activity was determined via tritiated thymidine ([(3)H]Tdr) incorporation studies of YYT colon cancer cells; the anti-angiogenic effect of gingerol was determined via Matrigel assays using MS1 endothelial cells. All in vitro studies show that 6-gingerol has an antitumor effects with a 2 ways: 1) direct way, colon cancer cell growth suppression, and 2) indirectly, inhibition of the blood supply of the tumor via angiogenesis. This finding indicate that 6-gingerol can be used as a potential anticancer plant within the complementary treatment against to cancer and similar to our anticancer effect of SC and SLO in our study [15].

In another study; the effects of lidocaine on the C6 rat glioma cell line were searched. Annexin V/propidium iodide analysis and MTT assay applied and the result showed that lidocaine has an anticancer effect via increasing in the percentage of apoptotic and necrotic cells. Also light microscopy analysis on the ultrastructural level were applied as well. The results indicated that; this compound effected apoptotic cells percentage increasingly via suppressing effect on the fusion of autophagosomes with lysosomes. On the basis of this result; lidocaine could be used as an alternative therapeutic strategy in the treatment of cancer like our findings with SC and SLO [16].

In different study; an "alternative medicine" possibilities against to metastatic hormone-refractory prostate cancer (CaP) was searched. For this aim and determination of their antitumor activity; "BIRM" (biological immune response modulator; "Simple Ecuadorian Oral Solution: an extract of an Amazonian plant"), was used in vitro and in vivo studies with CaP cell lines and a tumor model. The cytotoxicity effect of BIRM was determined via using cell proliferation-inhibition and clonogenic survival assays. As a result; ıt was found that BIRM inhibited cell proliferation and clonogenic growth of the CaP cells was (IC(50) about 8.0 microl/ml). It means that; cell accumulation increased in the G(0)/G(1) phase by 33.8% and the proportion of cells decreased in S phase by 54.6%. The result showed that; apoptotic cell death was associated with activation of cell death-associated caspases in response to BIRM treating effect. According to findings; The BIRM plant extract contains antitumor compounds with potent antiproliferative activity against to prostate cancer cells similar effect of our study products as well [17].

In another study; the connection between adipose tissue and breast cancer takes attention and the importance was given into the possible role of apelin, another hormone of fat tissue, in breast cancer. There are few studies related to the relationship between apelin and cancer. Berta et al. searched the effects of apelin applied to the human lung cancer cell (NSCLC) both in vitro and in vivo. As a result, they found that; in vitro apelin application did not affect cell viability, but in vivo study, the apelin application increased the development of tumor cell via stimulating angiogenesis. Similar methods were used with our study [18].

Carvacrol, found in oregano plant, is used as a spice in food. A study was carried out to determine the apoptotic effects of carvacrol into transformed CO25 and 5RP7 cells and to evaluate the possibility of carvacrol as a chemotherapeutic agent. The results of this study showed that carvacrol has an cytotoxic effects onto these cells. The IC50 value was obtained as 0.04 mg / mL for 5RP7 cells and 0.1 mg / mL for CO25 cells. According to these values, the carvacrol caused morphological change in both cell types, and the appearance of the staircase of DNA, the indicator of the late apoptosis mechanism, was observed in cells with H-ras oncogene at or below IC50, but not in cells with N-ras oncogen. These results indicated that H-ras cells were more sensitive to carvacrol than N-ras cells. Similar results were obtained with SC and SLO, which were used as chemotherapeutic agents in our study [19].

5. Conclusion

In our cell culture study using Caco-2 cancer cell line and SC and SLO; the death of cancer cells in the most efficient manner was obtained when the SLO is administered at the 24th hour and 200mg dose; and SC is applied at 6 hours with 120 mg dosage.

6. Referances

1. Akgül E. Formaldehit maruziyeti sonucu rat akciğerinde oluşan oksidatif hasara karşı ve deneysel olarak oluşturulan kolon kanseri üzerine köpek balığı kıkırdağı ve köpek balığı karaciğer yağının (SLO) koruyucu etkisinin belirlenmesi. PhD Thesis. Afyon Kocatepe Üniversitesi Sağlık Bilimleri Enstitüsü, 2018, 140.
2. Akgül E, Dosay-Akbulut M. Use of Complementary or Alternative Medicine In Patients With Cancer In Turkey. Journal of US-China Medical Science. 2014; 11(3):159-167.
3. Dosay-Akbulut M, Akgül E. Shark Cartilage and Liver Oil Using Possibilities Against to the Cancer Formation. Türk Bilimsel Derlemeler Dergisi, 2014; 7(1):42-45.
4. Patra D, Sandell LJ. Antiangiogenic and anticancer molecules in cartilage. Expert Reviews in Molecular Medicine. 2012; 14:e10. doi: 10.1017/erm. 3.
5. Szostak WB, Szostak WD. Health properties of shark oil. Przegl Lek. 2006; 63(4):223-6.
6. Lewkowıcz N, Lewkowıcz P, Kurnatowska A, Tchorzewskı H. Biological action and clinicalapplication of shark liver oil. Pol Merkur Lekarski. 2006; 20(119):598-601.
7. Lacroıx M. Persistent use of “false” cell lines. Int J Cancer. 2008; 122(1):1-4.
8. Aydıntuğ YS, Mutlu İ. Hücre kültürü ve diş hekimliğindeki uygulamaları. GATA Sağlık Bilimleri Enstitüsü Diş Hekimliği Bilimleri Merkezi, Ankara, 2003, 2-22.
9. Baysal K, Serhatlı M, Adıgüzel Z. İleri moleküler hücre biyolojisi teknikleri eğitimi. TÜBİTAK MAM Gen Mühendisliği ve Biyoteknoloji Enstitüsü, Kocaeli, 2008, 5-14.
10. Mosman T. Rapid colorimetric assay for cellular growth and cytotoxicty assays. Journal of immunological Methods. 1983; 65:55-63.
11. Koyunoğlu F, Tekin S, Konar V, Sandal S. İnsan Meme Kanseri Hücre Serileri (MCF-7) Üzerine Apelin-13’ün Etkilerinin Araştırılması: In Vitro Bir Çalışma. İnönü Üniversitesi Sağlık Bilimleri Dergisi, 2013; 1:23-28.
12. Zeytinoğlu H, İncesu Z, Ayaz TB, Türk AO, Barutça B. Determination of genotoxic, antigenotoxic and cytotoxic potential of the extract from lichen cetraria aculeata (Schreb.) Fr. in vitro. Phytother Res. 2008; 22:118-23.
13. Bozcuk H, Özdoğan M, Aykurt O, Topçuoğlu F, Öztürk H, Ekinci D, et al. Urginea maritima (L.) Baker (Liliaceae) extract induces more cytotoxicity than Standard chemotherapeutics in the A549 non-small cell lung cancer (NSCLC) cell line. Turk J Med Sci. 2011; 41(1):101-108.
14. Gürbüz V, Yılmaz A, Gökçe Ö, Konaç E. The Apoptotic Effects of Cisplatin on Human Colon Cancer Cell Line (HT29). 2011; 24(2):100-105. Conference Paper. 9th International International society for the study of xenobiotics Meeting. DOI: 10.5472/MMJ.2011.01784.1
15. 15- Brown AC1, Shah C, Liu J, Pham JT, Zhang JG, Jadus MR. Ginger's (Zingiber officinale Roscoe)

inhibition of rat colonic adenocarcinoma cells proliferation and angiogenesis in vitro. Phytother Res. 2009; 23(5):640-5. doi: 10.1002/ptr.2677.
16. Izdebska M1, Hałas-Wiśniewska M1, Zielińska W1, Klimaszewska-Wiśniewska A2, Grzanka D2, Gagat M1. Lidocaine induces protective autophagy in rat C6 glioma cell line. Int J Oncol, 2018; doi: 10. 3892 /ijo.2018.4668. [Epub ahead of print]
17. Dandekar DS1, Lokeshwar VB, Cevallos-Arellano E, Soloway MS, Lokeshwar BL. An orally active Amazonian plant extract (BIRM) inhibits prostate cancer growth and metastasis. Cancer Chemother Pharmacol, 2003; 52(1):59-66. Epub, 2003, 7.
18. Berta J, Kenessey I, Dobos J, Tovari J, Klepetko W, Jan AH, et al. Apelin expression in human nonsmall cell lung cancer: role in angiogenesis and prognosis. J Thorac Oncol. 2010; 5(8):1120-9.
19. Akalın G, İncesu Z. The Effects of Carvacrol on Apoptosis of H-RAS and N-RAS Transformed Cell Lines. Turk J Pharm Sci. 2011; 8 (2):105-116.