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RESEARCH ARTICLE

THE INVESTIGATION ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF CAPSAICIN AND ALPHA LIPOIC ACIDON COLON ADENOCARCINOMA CELLS

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ABSTRACT

Althoughmanystudieshave focused on antitumoral properties of capsaicin and alpha lipoic acid, molecular mechanisms by which selectively effects are incompletely determined. In current study, we investigated cytotoxic and apoptotic role of natural compounds on colon adenocarcinoma cells. Capcaicin was evaluated for different concentrations at Caco-2. Cytotoxic effects of capsaicin and alpha lipoic acid on Caco-2 cells were determined by MTT. However, antioxidant activities of alpha lipoic acid were evaluated when used with capsaicin or separately groups. Caspase activities were measured by apoptotic assays. Capsaicin and ALA, alone or in combination, inhibited cell growth and promoted apoptosis. Capsaicin has significant cytotoxic and apoptotic effects at 150, 300µM concentrations while alpha lipoic acid has proliferative effects with more than 400 µM on Caco-2 cells. However, we observed these compounds activate caspase 8 using the mitochondrial and death receptor pathways to induce apoptosis. Further studies at molecular level are required to support our findings and to elucidate chemotherapeutic flavonoids on colon cancer. Extrinsic, intrinsic, and mitochondrial pathway procedures show how to use apoptotic mechanism considering by stimulation. According to our results, medical plants should be used with the correct concentration and duration of the cancer type and the stage.

INTRODUCTION

Cancer is one of the most important causes of death all over the world even if It is possible to prevent approximately 30% of cancer-related deaths. It is estimated that cancer deaths worldwide will continue to increase and 12 million deaths will be due to cancer in 2030 (1). Colorectal cancers are third frequency after lung cancer and prostate cancer in men; are seen as the third most common cause of lung and breast cancer in females(2).

The lack of protective substances due to unbalanced nutrition in the pathogenesis of colorectal carcinoma and the loss of regeneration resistance and mucus quality in colon mucosa epithelial cells caused by carcinogenic factors have been reported that carcinogenesis begins as a result of its persistence with genetic and somatic mutations. The rapid increase in information about the molecular and biological properties of colorectal cancer provides an explanation for the interaction between genetic susceptibility and environmental effects(3).

Colorectal carcinogenesis occurs as a result of the accumulation of genetic changes that lead to the disruption of the molecular mechanisms that control growth in normal tissues (4). Colon cancer is a consequence of molecular events that are caused by irregular DNA replication and colonization of genetic mutations that occur in cascades. These genetic events happen diminution or loss of tumor suppressor genes, activation of protooncogenes and mismatch repair mechanisms (5).

Although colorectal cancer treatment is possible in early stages, the mortality rate is high since tumor symptoms usually occur in advanced stage. Biomarkers are frequently used to determine cancer risk. The main purpose of using biomolecules is; Early diagnosis in cancer diagnosis, determination of long term tumor size and activity, evaluating the treatment process and having knowledge about the factors causing toxicity (6).

Flavonoids are polyphenolic compounds and are phytochemicals that are popular due to antioxidant and free radical scavenger effects. These compounds may be used to treat oxidative DNA damage; further researches show as well as protection and strengthening of endogenous antioxidants (7). Phytochemicals are important for detoxifying enzyme activation, immunostimulatory, gene expression related to cell

proliferation and apoptosis, hormone metabolism, antibacterial and antiviral effects (8).

Chemopreventive agents are divided into two main categories as blocking and suppressing agents, according to the developmental stages of cancer cells. Blocking agents act as inhibitors of carcinogens that interact with important cellular macromolecules such as DNA, RNA, protein, to reach target tissues. On the other hand suppressor agents inhibit the conversion or progression of cells in their initial stages into the malignant form. Cellular and molecular events such as DNA repair, cell cycle, cell proliferation and differentiation, apoptosis, functional activation and expression of oncogenes and tumor suppressor genes, metastasis and angiogenesis, effects of hormonal and growth factors can be regulated by chemopreventive phytochemical containing agents (9,10). The data obtained from epidemiological studies show that some of the herbal medicines taken with the diet are cancer-inhibiting properties of flavonoids such as resveratrol in red grapes, genistein in the syrup, curcumin in turmeric, capsicum in pepper, propolis in honey, catechin in green tea, lycopene in tomato, quercetin, hesperetin in citrus (11, 8, 12).

Chili peppers contain a plant compound known as capsaicin, which is one of the most important members of food through its painful and stimulating effect. This effect is the species belonging to the genus Capsicum of the Solanaceae family, commonly known as the capsaicinoid. Due to their irritating properties, other studies have shown chemopreventive and chemoprotective effects, although not considered about carcinogenic or co-carcinogenic role in experimental animals (13, 14, 15).

Dietary phytochemicals are low cost, easy to apply and preferred chemopreventive approach according to their effect for cancer prevention and control. However, it has been found that flavonoids are significant differences between in vitro bioavailability and in vivo bioactivity. Therefore, before anticarcinogenic and apoptotic effects of flavonoids are evaluated, its metabolism, absorption and bioavailability should be examinated (11,16).

The capsaicin molecule from Capsicum annuum was first isolated in crystal form by Bucholz in 1816. The content of capsaicin ranges from 0.22 to 20 mg /g in different pepper species (Capsicum frutescens, annuum and chinese). Researches have shown that red pepper consumes 2.5 g per person per day in India, 5 g in Thailand and 20 g in Mexico (17). The form of capsaicin molecule, called 8-methyl-N-vanillyl-6-nonenamide, is the closed formula C18H27NO3 and is an odorless and colorless powder. It has maintained the original structure of capsule despite waiting, freezing and baking. Capsaicins are straight-chain alkyl vanillylamides which are highly stable phenolic. Capsaicin biosynthesis occurs due to the AT3 gene, which suppose to encode acyltransferase enzyme (17,18). Capsaicinoids are absorbed 85% intragastrically, metabolized in the liver before reaching the general circulation and extra hepatic organs (19). Capsaicin, which acts as antioxidant, strongly inhibits lipid peroxidation but their mechanism do not reveal clearly. It has been reported that capsaicin effectively destroys DPPH radicals and OH-radicals in ethanol and membranes, the effect level is similar to a-tocopherol, and that molecular agent destroys the 2-molecule DPPH radical. It is stated that antioxidative effect is related to the settlement site in the membrane, and capsaicin is the most effective agent for the reactive oxygen species near and at the membrane surface (20). Capsaicin-induced apoptosis is thought to be caused by oxidative stress-related damage to cellular macromolecules such as protein, lipid, DNA and RNA, with still controversial (21).

A-lipoic acid, which is the only antioxidant soluble in both oil and water, is also called 6,8-dithiooctanoic acid. Alpha lipoic acid is a natural substance found in exogenous foods and at the same time synthesized in the body. The biosynthetic pathway of lipoic acid occurs from ocanoic acid and a sulfur source in eukaryotes mitochondria, and is produced mostly in the liver (22). LA is a low molecular weight substance that can cross the blood -brain barrier (23). Lipoic acid is absorbed in 93% excess when given orally, with first-pass effect 20-30% about metabolism in the liver. The beneficial effects of LA were determined at a plasma concentration of 10 μM and the maximal effect was observed at 300 μM (24).

Lipoic acid, which plays an important role in mitochondrial dehydrogenase reactions, has recently been noted as an important antioxidant. Lipoic acid also protects the membranes by interacting with Vitamin E, which regulates Vitamin C and glutathione. Lipoic acid has been shown to be beneficial in some of the oxidative stress models such as diabetes, ischemia-reperfusion injury, cataract formation, HIV activation, nerve degeneration and radiation damage. In addition, acts as a reducing regulator of proteins such as myoglobin, prolactin, thioredoxin, and the NF-κb transcription factor (25).

Lipoic acid has only antioxidant for protective effects on both the reducing and the oxidation forms. LA plays a central role in the antioxidant system, can reduce all antioxidants and regenerate lipoamide reductase, glutathione reductase and thioredoxin reductase enzymes, and increase glutathione (GSH) levels at treatment (26). ALA and the reduced form of DHLA are strong antioxidants, with the specificity to remove free radicals, the interaction with other antioxidants, the metal shaving activity, the effects of gene expression, the bioavailability and the ability to repair oxidative damage (27).

Oxidant molecule is uptake which can be up to a certain level, is neutralized by natural antioxidants which are also found at a certain level in the body. If this balance might be impaired, there is an increased risk of aging, cell destruction and tumor formation, especially oxidative stress (28,29).

Apoptosis is time-lived killing under programmed, genetic control of the life cycle of infected or undesired cells with energy using harmful, aging, bacterial and autoreactive viruses. In normal cell, 7 consecutive breaks repairing although 300,000 breaks occur in the apoptosis and cell repair is not possible (30).

Apoptosis is regulated by intracellular and extracellular mechanisms by a wide variety molecules. Caspases are enzymes of the cysteine-protease group, which consists of the multigen family that plays an important role during apoptotic cell death (31). Since the different tissue type stimulates different caspase pathway, apoptosis take place through the activation from different caspases mechanism. The apoptotic process has been initiated by extracellular or intracellular mechanisms, there is also is carried out by proteolytic enzymes called caspases. Caspases are play an important role in apoptosis activation function by 3 different ways(32).

Extrinsic, intrinsic, and mitochondrial pathway procedures show diversity according to the stimulation of apoptosis mechanism of the cell cycle. Tumor cells acquire an apoptosis-resistant property by overproduction of antiapoptotic proteins or reduction of the production or effects of proapoptotic proteins. Caspase inhibitors or activators are effective at distinct steps of cell death, therefore are being tested for therapeutic use(32) Cancer treatment is aimed to kill tumor cells with minimal damage to surrounding normal tissue. Apoptosis (programmed cell death) is a physiological process that allows cells to be killed without producing an inflammatory response and is therefore widely used in cancer treatment (33).

Cancer mortality rates are increasing every year despite early diagnosis and therapeutic advances. Although the in vivo and in vitro anti-inflammatory and anti-oxidant effects of capsaicin are well known, the molecular mechanism of capsaicin function in human colon cells (Cacos) stimulated by ALA (alpha lipoic acid) remains unclear. The administration of antioxidants has been shown to alter the interaction modes of cell cycle that play a role in carcinogenesis(34).

Researches on the use of capsicin which is active ingredient of red pepper are being carried out.cancer treatment. The effect of alpha lipoic acid on colon cancer, which is one of the most important antioxidants for our study, has been known from previous studies. We are aimed to explain the mechanism of action of capsaicin and alpha lipoic acid which are unique for our study in colon cancer cells, demonstrate which way they use, their antiproliferative and apoptotic properties using different methods. For this purpose, caspase 3 as main cascade on apoptotic pathway, caspase 8 for intrinsic (mitochondrial) pathway and caspase-9, annexin V and DNA laddering analysis for external stimuli were performed in Caco-2 cells treated with capsaicin and alpha lipoic acid.

MATERIALS AND METHODS

Materials and chemicals

Capsaicin, ethylenediaminetetraacetic acid (EDTA), DMSO, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St.Louis, MO, USA). Medium 199, DMEM F12, Fetal bovine serum (FBS), and Trypsin–EDTA were obtained from Lonza (Switzerland). DNA ladder kit, Caspase-3, Caspase-8, Caspase-9 colorimetric kits, %1 Non-essential aminoasit(100X) were purchased from Millipore Corporation (Billerica, MA, USA). TNF-α and COX-2 elisa kits were provided from Biolegend company. Cell culture consumables were purchased from BD Transduction Laboratories (San Diego, CA, USA).

Cell cultures

Human colon adenocarcinoma cells (Caco-2) were purchased from ATCC (ATCC# CRL-1730; Manassas, VA) and passages 5–9 were tested for experiments. The cells were removed from the cryostats stored at -80°C and quickly thawed at 37°C in a sterile water bath. The tubes were removed with 70% alcohol and taken into the laminar flow cabinet to avoid possible contamination. Cells were dispersed gently by pipetting and cultured in DMEM F12 (Lonza, Switzerland) culture medium containing 20% Fetal Bovine Serum (FBS) (Lonza, Basel, Switzerland), 1% Penicillin /Streptomycin, 1% NEA. All cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO2.

Treatment of Cacos with Capsaicin and ALA

Twenty-four hours prior to administration, the cell suspension was transferred to 6 well growth vessels and allowed to incubate for 24 hours at 37 °C in a 5 % CO_2 incubator. DMEM medium was then removed from the culture medium and replaced with 5 ml of fresh medium at 25 µM (total volume 1/500), 50 µM, 150 µM and 300 µM concentrations of capsaicin were applied of Caco -2 cells for 6 h, 24 h and 48 hours. For other groups of cells were produced to administer alpha lipoic acid; the alpha lipoic acid was added to the media at concentrations of 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL in the same manner. However, only DMSO solution was added to a cell group in the same volume instead of the experimental group using as a control.

Since no significant changes were observed in the groups of cells that we left in 6 hour incubation, there was no 6 hour application were made for other experimental procedures.

Cell viability assay

Caco-2 colon carcinoma cell lines, which reached 80-90% density in 75 cm2 flasks, were determined by counting on thoma slide. The number of Caco-2 cells confluent about 70-80% in the T75 flask was calculated to be $1.67x10^6$ /ml. Caco-2 cells were seeded in 96-well plates in an equal volume of $5x10^4$ cells /100 µl in each well to assess cellular function. The concentrations of Capsaicin were applied as control, 25 µM; 50 µM; 150µM and alpha lipoic acid concentration 50 µM; 100 µM; 200 µM; 400 µM, also combined therapy were applied as shown in Table 1. Then MTT test was specifically used in our study for the detection of viable cells in cell proliferation and cytotoxicity. (Table I)

Table I Cultureapplicationdoses

Cultureapplicationdoses		
Capsaicindoses	**Alpha LipoicAciddoses**	**Caps+ALAdoses**
300 uM	400 uM	300 uMcaps +200uM ALA
150 uM	200 uM	150 uMcaps +200uM ALA
50 uM	100 uM	50 uMcaps +200uM ALA
25 uM	50 uM	25 uMcaps +200uM ALA
DMSO control	DMSO control	200 uM ALA
DMEM control	DMEM control	DMEM control

Caspase activities

The cell lysates of experimental groups were prepared to equal protein amounts. For this purpose, BSA test has performed on different concentrations of applications.

Caspase activity was determined using a colorimetric assay based on the ability of caspase-3 to change acetyl- Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) into a yellow formazan product (p-nitroanilide (pNA)). An increase in the absorbance at 405nm was used to quantify the activation of caspase activities. $0.5\text{-}2X10^6$ cells were prepared for colorimetric analysis. The cells were rinsed with cold PBS and then were lysed with lysis buffer for 15 min on ice. The cell lysates were centrifuged at 18,000 g for 10 min at 4 °C. Caspase activity in the supernatant was assayed using the colorimetric kit. Caspase activity was expressed as a percentage of the enzyme activity compared with that of the control.

DNA pattern

Internucleosomal DNA fragments that occur during the late phase of apoptosis shows that a typical ladder step image on agarose gel electrophoresis. Therefore, the DNA fragments isolated from the cell groups were used in the DNA ladder kit to determine the apoptosis stage in the cells. Apoptotic DNA ladder commercial kit (Chemicon, Temecula, CA, USA, APT151) was used for DNA precipitation then the ladder formation of the DNA breaks were demonstrated by loading a 1% agarose gel.

Annexin V staining

In normal cells, there is phosphatidylserin (PS), which one of membrane lipids on cytoplasmic surface of the cell membrane. If the cell goes apoptosis, the PS molecules normally located on the inner surface translocate to the outer surface of the cell membrane. This migration occurs during the early stages of apoptotic cell death, where cell membrane integrity has not compromised yet. The annexin V protein which is Ca^{+2} dependent, 35.8 kDa weight, can bind specifically to FITC stain, determines early apoptotic signals in living cells.The metabolic activity in the cells were measured using ApopNexin™FITC apoptosis detection kit (Chemicon, Temecula, CA, USA, APT750). 10^5-10^6 of apoptosis-induced cells were taken and protocol was applied. Healthy cells and FITC-stained apoptotic cells were visualized on a fluorescence microscope (Olympus, Japan).

Statistical analysis

The descriptive values and the standard deviation are given according to the quantitative measurements obtained in the study. The One-Way Anova method was used to compare the groups in terms of the averages of the measurements. Dunnett's and Tukey tests were used as post-hoc tests for further comparisons of different groups. The mean comparisons of the two groups were analyzed by student t test. Statistical significance was 0.05 and p <0.05 was considered as statistically significant. SPSS (version 21) program was used for calculations. (Sigma-Stat 2.0, Jandel Scientific, San Rafael, CA, USA).

RESULTS

Viability change of Caco-2 cells incubated for 24 h, 48 hours with 0, 25, 50, 150, 300 µM capsules used as positive control in all experiments were determined by MTT viability test. No treatment µM of control (control) was taken as 100% for all time periods, and the viability values of the other samples were calculated from the mean absorbance values. % Viability rates in cell lysates were markedly decreased compared to increasing concentrations of capsaicin.(Table II)

Data resultsaccordingtoconcentration					
MTT Results(nm)	**Control (DMSO)**	**1.Dose (25ug/ul)**	**2.Dose (50ug/ul)**	**3.Dose (150ug/ul)**	**4.Dose (300ug/ul)**
24 h	1,187	1,086	1,007	0,877	0,807
48 h	0,959	0,768	0,695	0,623	0,427

Table II MTT variation

MTT concentration/ P value		
(I) VAR00001	**(J) VAR00001**	**p**
caps25	control	1,000
caps150	control	0,909
caps 300	control	<0,001

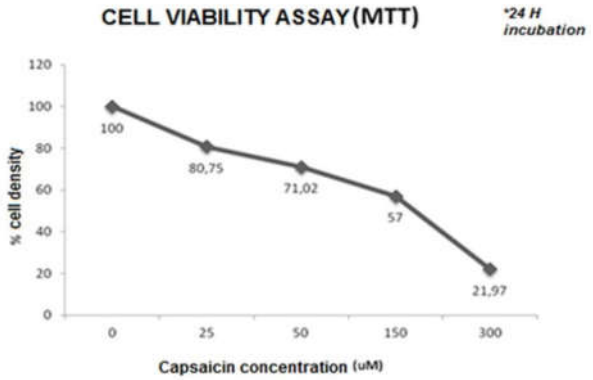

Figure I Percentviabilityrates in capsaicin-treatedcells (24 hours)

However, the value of the effect increased in cells subjected to the dose-dependent (0; 25; 50; 150; 300 µM) incubation for 48 hours incubation as shown in figure 2 and cell viability decreased by 88.24% compared to the control.

As a result of application of alpha lipoic acid, which acts as both pro-oxidant and natural antioxidant, Caco-2 cell viability was increased by 4.84%; 13,24%; 17.73%; 39.47% for 24 hours as dorsal-dependent (50; 100; 200; 400µM), is indicated in figure III. (Figure II)

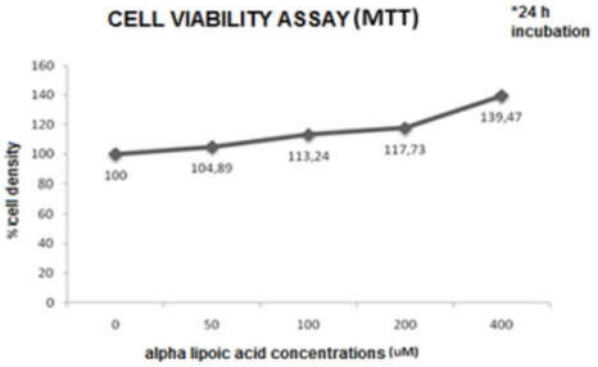

Figure II Percentviabilityrates (24 hours) in Caco-2 cellstreatedwithalphalipoicacid

In the light of these results, alpha lipoic acid, which acts as an antioxidant, has also been shown to induce cell viability. Despite the cytotoxic effect of capsaicin flavonoid in cell viability, the proliferative effect of alpha lipoic acid supports the idea that it may exert antagonistic effect on capsaicin administered group in caspase activities.

Cell viability rates read at spectrophotometer (560 nm) at the 24 hour and 48 hour incubation of different doses of capsaicin with the optimal dose of alpha lipoic acid application (200 uM) determined on colon adenocarcinoma cells are given in table 2.It has been found that the co-administration of capsaicin and alpha lipoic acid reduces the cytotoxic effect of capsaicin on cells. This decreasing effect is an expected result due to the proliferative effect of alpha lipoic acid.

It was observed that capsaicin molecule administered at 300 µM dose for 48 hours increased the activity of caspase 3 which is involved in cell apoptosis mechanism at a statistically significant level.In association with the proliferation of Caco-2 cells, caspase-3 activity was found to decrease at high dose LA exposures.(Figure III) Alpha lipoic acid exhibited the desired activity with optimal dosage (200 µM) for cell proliferation and 25; 50; 150; 300 µM doses of capsaicin were administered to the cells for 48 hours.

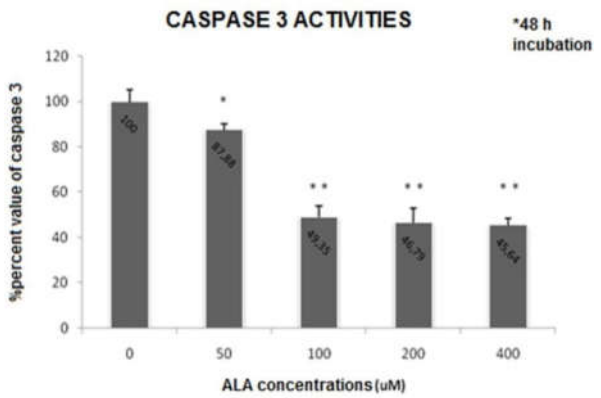

Figure III Caspase 3 activities in theintracellularlyadministered ALA to Caco-2 cellgroup

At the end of the incubation period, Caspase 3 activity was observed to be lower in cell group compared to the group in which capsaicin was administered alone. Caspase 3 activities in combination with capsaicin and alpha lipoic acid are also compatible with the results of the obtained cell viability test. (Figure IV)

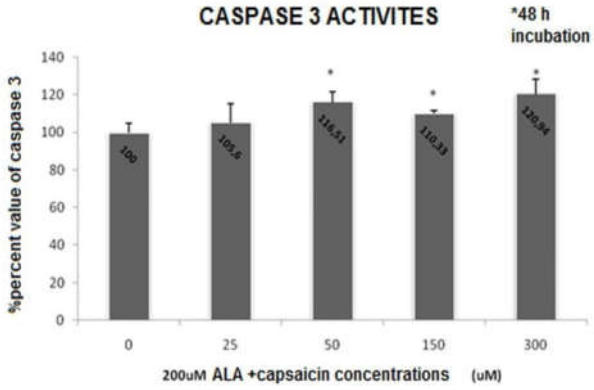

Figure IV Caspase 3 activities in the intracellularly administered combined to Caco-2 cellgroup

Activation of caspase-8 cascade of capsaicin molecule suggests that apoptosis mechanism is initiated via extracellular receptors.(Figure V)

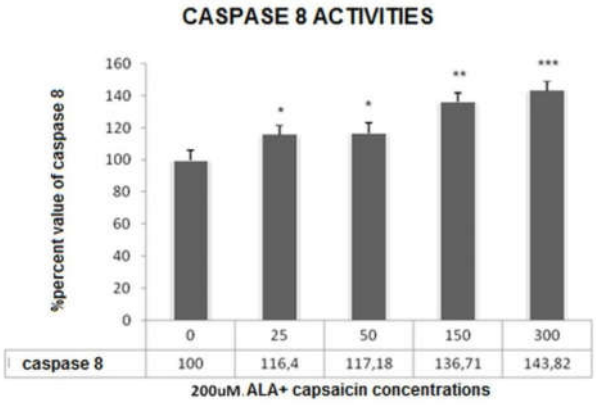

Figure V Caspase 8 activities in theintracellularlyadministeredto Caco-2 cell group

Caspase 8 activities were examined in the cell group in which alpha lipoic acid and capsaicin were co-administered. Where two molecules were acting together increase caspase-8 signal was less than single capsaicin treatment.

Caspase 9 is a critical step that is active in the intrinsic signal pathway that directly affects intracellular receptors. Caspase 9 activities are important for determining the initiation signals on the mitochondrial pathway in the apoptosis mechanism. In the study, increasing doses of caspase 9 activity in the cell group given capsaicin when compared to control 48-hour incubation.(Figure VI)

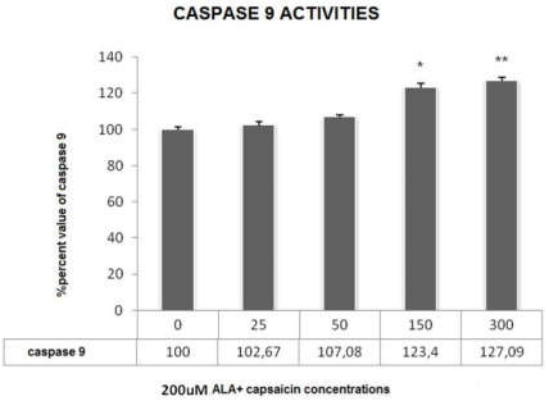

Figure VI Caspase 9 activities in the intracellularlyadministered to Caco-2 cell group

Apoptotic changes initiated by stimulation of intracellular pathways are mediated by the expression of pro- and anti-apoptotic proteins and the induction of caspase cascades. At the highest dose of combined use, caspase-9 activity was statistically insignificant with an increase of 27.09%. It was also found that co-administration of capsaicin and alpha lipoic acid was less effective than single administration.

Capsaicin and alpha lipoic acid were subjected to DNA laddering test to determine late apoptotic effects on Caco-2 cell. Accordingly, DNA fragments were not formed at concentrations of 25 µM, 50 µM capsaicin. 150 µM and 300 µM capsaicin application showed similar ladder formation to staircase stones indicating increased DNA breaks. There was no significant change in apoptotic activity in the group of cells cultured for 48 hours with the variable dose alpha lipoic acid. (Figure VII)

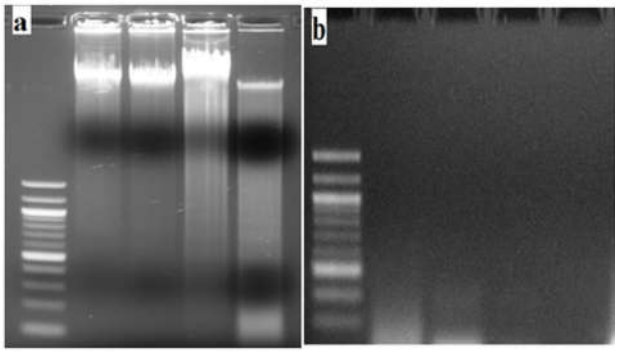

Figure VII DNA ladderingagarose gel electrophoresis of Caco-2 cells in treatmen tgroups
(a-capsaicindoses experimentgroup, b-ALA dosesexperimentgroup)

The annexin V test, in which the apoptotic cell was visualized by fluorescence in the early phase, was assessed by fluorescence microscopy. The number of apoptotic cells marked with green-fluorescent substance (FITC) in the cell group of the capsaicin-treated group increased significantly compared to the control cell group in Figure XI (Figure VIII)

DISCUSSION

The six characteristics that characterize from Hanahan and Weinberg team, Cancer cells are self-replenishment in growth signals, insensitivity to signals that can stop growing,

apoptosis-free survival, unlimited division, possession of proliferative potential, sustained angiogenesis, tissue invasion and metastasis (34).

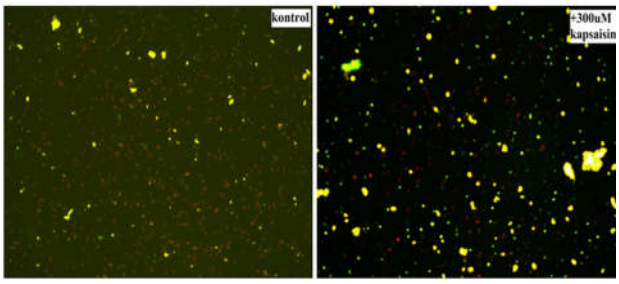

Figure VIII Aponexin V fluorescencemicroscopeimages of capsaicin-treated Caco-2 cells (x100)

The flavonoids that set up our work, is known that regulation of the nitric oxide (NO) synthesis in the vascular tissue, increasing the intercellular communication, inhibiting cell proliferation by inhibiting the incorporation of thymidine decarboxylase and thymidine into the DNA construct, stimulating antiproliferative effect by blocking the respective receptors, inhibiting lactate transport and inhibiting calmodulin leads to apoptosis of the cells and an antitumor effect(35).

The results from some studies support the idea that the capsaicin compound, a red pepper extract, has carcinogenic effects, and some studies show that the capsaicin compound is a tumor-inhibiting property (35,36).

This double-edge sword effect is not related to the waning of capsaquinoid compounds. Because studies have shown that peppers that are not brackish and brackish can show the same antioxidant effect. Capsaicin molecules can break free radicals both on the cell membrane and in the cell (37).

Capsaicin and other members of the capsaicinoid group have effects on the gastrointestinal system, cardiovascular system, respiratory system, limbic system and thermoregulator system. Capsaicin causes a permanent increase in blood pressure and an arrested respiratory arrest in the cardiovascular system, depending on dose and administration rate. Capsaicin treatment is indicated in the symptomatic treatment of muscle and joint pain, arthritis. peripheral neuropathy when applied 0.025-0.075% topically. That stimulate neuronal calcium channel blocker in nervous system. Curative effect was observed by inhibiting the release of neurotransmitters from neurons to result in less vasodilatation, plasma extravasation, less histamine / serotonin secretion, and neurogenic inflammation.

Epidemiological data suggest that obesity rates are reduced due to the intake of capsicain-containing foods. It has been found that has a protective effect against experimentally induced gastric damage during administered capsaicin intragastrically. This effect may be due to increased gastric mucus production and decreased mucosal mucus consumption by inhibiting gastric acid secretion by vagal nerve inactivation. Capsacin has some effect on the transport of some electrolytes in the intestinal tract and on peristaltic activity. This effect results in the interaction with the TRPV1 receptor present in the capsaicin receptor or gastrointestinal channel stimulated neurons. Some dietary components and phytochemicals such as capsaicin have recently been shown to be important factors influencing drug absorption, distribution and elimination(37).

Capsaicin molecule, the active ingredient of red pepper, has been shown to improve apoptosis rate in cancer cells while being positive results like many diseases with supported by studies. Natural capsaicin have been shown to inhibit growth myeloid leukemia, lung cancer cells, prostate cancer cells, human hepatoma HepG2 cells and colon cancer cells by inducing apoptosis (37,38,39).

Gálvez and colleagues examined the cytotoxic effects of red pepper extracts of the genus Capsicum, used as traditional medicines for cancer treatment, against three human cancer cells. Capsicum annuum has been identified as a strong flavonoid in many Solanaceae species since flavonoids can exhibit a potent inhibitory effect on the proliferation of human cancer cells. In recent research, capsaicin acts to increase apoptosis in human melanoma cells throughsuppresses the expression of the hippocampus-activated DNase inhibitor, reduces the intracellular antiapoptotic Bcl-2 ratio and increases caspase-3 activity. Relation to this, the capsaicin increases intracellular reactive oxygen species and calcium level at carcinoma cell type(40,41) It is thought that the anticancer activity of capsaicin molecules is caused by apoptosis stimulating effect. Capsaicin's cell-death stimulating effect is usually mediated by oxygen sensitive molecules, which are usually the result of the action of capsaicin molecules on the mitochondrial electron transport system (41).

On the other hand, capsaicin is also reported to be at risk for certain strains such as stomach cancer. The cancer protective effect of Capsaicin is controversial, however that may be a cancer predisposing factor shows with epidemiological studies. These are important factors in usage of dose, duration and type of cell for the direction of bi-directional effects (41,42).

In recent studies, antianjiogenic activities have been demonstrated in in-vitro and in-vivo systems rather than antigenotoxic, antimutagenic and anticarcinogenic effects. It prevents VEGF-induced angiogenesis signaling pathways by stopping the G1 cell cycle in endothelial cells. Thus, the molecule prevents the formation of metastases accordingly the morphological changes in cells, the migration of malignant cells and the antiproliferative effect in the blood vessels (43).

The TRPV1 gene which activated from capsaicin reduce malignancy and control tumor progression in adverse ways at bladder cancer and brain tumors. Recent studies support the activation of TPRV1 protein, acting as a tumor suppressor gene in skin cancers previously.

Epidermal growth factor receptor (EGFR) is tyrosine kinase receptor where overexpressed in colorectal, pancreas, lung cancer that regulate intracellular cascades such as Ras / Raf / MEK / ERK and phosphoinositide-3-kinase /Akt which control proliferation, migration, apoptosis mechanism. Capsaicin increases EGFR-dependent COX-2 expression and acts as a tumor suppressor in this signaling pathway. (Szallasi ve ark., 2007; Hwank ve ark., 2010).Caspase 3 activity increases due to DNA fragmentation using DHLA in different doses of 24 hours in colon cancer cells. Also mitochondrial O2 production accelerates mitochondrial flow of lactate or pyruvate. This results in the suppression of antiapoptotic proteins such as Bcl-xL, in this manner apoptosis is induced by the effect of ALA and DHLA(44,45).

Capsaicin show variable carcinogenic potential results in vitro and in vivo which are evaluated by genotoxicity and carcinogenesis analyzes. However, since 2000, the standardization of protocols show that the carcinogenicity of capsaicin is very weak and its purity is important. The

classification of capsaicin as an important therapeutic agent is due to the antiproliferative effect on cancer cells in cancer cell lines and xenograft mouse models, the arrest of the cell cycle in the G0 / G1 phases and in particular the induction of tumor cell apoptosis. However, it is unclear which molecular mechanisms induce apoptosis. Possible causes of capsaicin-induced induction of apoptosis include that irreversible oxidative stress-induced irreversible damage, back regulation of NFkB activity, forward regulation of proapoptotic proteins, caspase activation of the intrinsic (mitochondrial) pathway and irregularity of the ubiquitin / proteosome pathway (46).

Tsou et al.researches indicate that induce apoptosis by reducing the mitochondrial membrane potential due to an increase in reactive oxygen species (ROS) and Ca+2 production in human leukemia HL-60 cells. Apart from leukemia cells, it has also been shown that the growth of various cancer cells is inhibited such as melanoma, breast, adenocarcinoma, hepatocellular carcinoma treated with pure capsaicin (46).

Studies by Maity and colleagues published in 2010 have shown an important trail in the elucidation of the molecular mechanism by showing that apoptosis is induced by the degradation of cellular proteasome function via ubiquitin /proteasome pathway on mouse neuronal cell lineage (47).

Capsaicin administered in HT29 colon cancer cells was found to induce cell death through the peroxisome activator receptor without being dependent on the vanilloid receptor. Previous studies have shown that capsaicin-induced colon cancer increases the expression of ribosomal protein by weakening the transepithelial connections in the Caco-2 cell line. It has also been reported that the application of capsaicines in increasing concentrations of 100 µM-1 mM in Caco-2 cells results on decreased epithelial barrier functions due to the polysaccharide. Associated with this, it is understood that the activity observed in the use of capsaicin varies depending on the dose and duration (48).

Caspase-dependent or independent apoptosis induction in caspase-treated cells and determination of the apoptotic pathway used by them are investigated by measurement of protein expressions bound to receptors or by caspase activities. Apoptotic cell death for experimental HCT116 colon cancer cell occurs both extrinsically and intrinsically. The results of the study suggest that the increase in caspase-8 activity, which is responsible for the extrinsic pathway, and caspase-9 activity, which is responsible for the intrinsic pathway, suggests that apoptosis progresses from both mechanisms.

Our findings in study show compliance with previous studies. Caspase 3, caspase 8, caspase 9 have been shown to induce apoptotic pathways in cells applied cell group intracellularly. At the same time DNA fragments seen in the apoptotic phase were seen at a significant level in this group. The caspase-3, which is the initiator caspase-3 first in cells incubated with capsaicin for 48 hours, then coincidentally increases the caspase-9 activity and caspase-8, which are active in the extrinsic pathway, supporting cell death by using both pathways.

Previous studies indicate about the prooxidant and antioxidant propery of alpha lipoic acid showed increased mitochondrial respiration and a strong radical superoxide anion (O2-) level in HT29 colon cancer cells although the same effect was not observed in normal cells. In a similar study, ALA showed a minimal effect on the number of normal cells, and apoptosis was detected on mesenchymal tumor cells such as Jurkat, FaDu, Ki-V-Ras (49).

In our work, cell proliferation observed in alpha lipoic acid administration groups may be due to the proliferative effect of ALA. However, in the alpha lipoic acid-administered group, the levels of caspase are lower than those of the control group due to the prooxidant properties of alpha lipoic acid. DNA fragments were not observed significantly in the DNA laddering test, as it was known that alpha lipoic acid induced cell regeneration when applied in vitro. This suggests that the proliferative effect of ALA reduces apoptotic death in cells. The increasement in cell viability as the desire of applied ALA increases confirms with opinion.

The differences in caspase activities between the ALA-single applied and the co-administered (capsaicin+ ALA) cell group in the study results suggest that capsaicin molecule is caused by the suppression of the cytotoxic effect on the cells by alpha lipoic acid. The requirement for combined use promote the alpha lipoic acid protective effect against capsaicin single application for cell degeneration and rapid apoptosis.

In our study, we determined that capsaicin causes significant statistical changes in cell division and cell death after 24 and 48 hours compared to the negative control in all experimental group. However, alpha lipoic acid has been shown to stimulate cell proliferation in colon cancer as well as antioxidant properties. Combined therapy with ALA on capsaicin causes cell death to be slower. The cytopathic effect on cells in apoptosis mechanism was determined by the caspase activity which pathway was used. The essential features of in vitro studies are the determination of safety and efficacy of flavonoids taken from the external for adjuvant therapy before in vivo use. Advanced molecular studies have been needed in order to use capsaicin and alpha lipoic acid more widely in the treatment of colon cancer.

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