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Cytoxic Effect Levels Of Sheep Whey Protein in Colorectal Adenocarcinoma Cell Line (Caco-2)

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Abstract:

Objective: *It was aimed to determine the antitumor and antiproliferative activity of whey protein (whey) obtained from sheep colostrum isolation on colorectal cancer cells (Caco-2).*

Method: *Colostrum was separated into fractions as whey proteins using the isoelectric point. Before cell culture was studied, whey protein was sterilized by a membrane filter. Antitumoral activity levels of different concentrations of lyophilized proteins were measured by MTT viability test on cells and IC50 values were determined.*

Results: *Sheep whey proteins were incubated with Caco-2 cells for 24, 48 and 72 hours with serial dilutions starting with 3200 µg/ml and decreasing. When incubated with sheep whey Caco-2 cells, cell viability: 66.34% at 24 h; IC50 value of 8.414 µg/ml; IC50 value of 46.61% at 48 h was 5,838 µg/ml; The IC50 value of 4.61% at 72 h was found to be 4,741 µg/ml.*

Conclusion: *It was concluded that whey proteins obtained from sheep colostrum isolation have apoptotic and anticarcinogenic effects on Caco-2 cancer cell line and significantly inhibit the growth of tumor cells. We think that the results of this study will lead to studies to be carried out in vitro.*

Keywords: Colostrum, colorectal adenocarcinoma, cytotoxicity, whey proteins

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Introduction

Colorectal cancer (CRC) is the third most common cancer type in the world after lung and breast cancer. Every year, approximately 608,000 deaths occur due to CRC in the world[1]. Colorectal cancer usually results from its progressive accumulation with genetic and epigenetic differentiation that transforms normal colon epithelium into colon adenocarcinoma. Chromosomal instability, oncogenic mutation of RAS and BRAF genes, familial predisposition, smoking, age and diet can be counted as causes of colorectal cancer[2]. The composition of colostrum and the substances it contains vary depending on the animal's feeding, environmental and housing conditions, when colostrum is collected from the breast in the postpartum period, and the processing and condensation procedures of colostrum[3]. Along with cystine, colostrum has a rich content in terms of albumin, lactoferrin and lactoalbumin proteins, which plays a role in the transport of iron and copper into the cell[4]. It has been reported that the proteins present in colostrum form a chelate with iron, exert a protective effect in the cell against lipid peroxidation of iron, have a protective effect on the cell in its content, and show anticarcinogen and antioxidant effects due to the conjugated linoleic acid in its content[5]. It is stated that colostrum is an extremely safe natural nutrient, and it has no significant side effects other than mild gastrointestinal complaints such as bloating and nausea in humans[6]. Colostrum consumption by the offspring of ruminant species (cows, sheep, and goats) has a fundamental role in passive immune transfer and neonatal survival[7]. However, it directly affects the immune level of the lamb in the body immune system and plays an important role in protecting the animal from microorganisms[8]. Colostrum quality; It is under the influence of many factors such as the age of the animal, breed, nutritional level before pregnancy, length of stay in the dry period, difficult birth, size and behavioral factors[9].

Materials and Methods

The colostrum sample used in the study was obtained from sheep (Akkaraman) under hygienic conditions. Sheep colostrum was obtained by milking on the 3rd day. The milk was delivered to the laboratory by cold chain. The study was carried out in Dicle University Health Research Center Laboratory. The pH was adjusted to 7.6 if 1 M NaOH was added to the degreased colostrum samples. Then, whey protein was obtained from the supernatant by centrifugation at 4000 g for 30 minutes at 15 °C. Phosphate Buffer Saline (PBS) 3 times the volume taken from the obtained whey proteins was added and centrifuged at 4000g for 10 minutes at 15°C and washed. After washing, whey proteins were purified from microorganisms by passing them through a 0.22 µm membrane filter with the help of Millipore vacuum pump. Colostrum samples, which turned

into powder after 96 hours of lyophilization, were stored at -80ºC until the time of study. In our study, the MTT test, which is one of the cell viability analyzes, was preferred. Whey protein was weighed on a precision balance of 0.032 g and dissolved in 1% PBS (1 ml). After being completely homogenized, it was prepared in sterile eppendorf tubes with serial dilution at different concentrations in a laminar flow safety cabinet. 3.200 µg/ml, 1.600 µg/ml, 800 µg/ml, 400 µg/ml and 200 µg/ml of whey proteins were added to 96-well plates in equal amounts to each well by serial dilution. Then, incubation process was performed for different durations such as 24, 48 and 72 hours. After incubation, 10 µl of MTT solution (5 mg/ml) was added to sterile wells and kept in an incubator containing 5% CO2 at 37 °C for 3 hours. After the incubator, 100 µl of DMSO was added to the wells with the help of a multi-pipette in order to dissolve the formason crystals, and the 96-well plate was covered with aluminum foil and shaken for 10 minutes. At the end of the time, absorbance was measured with a microplate reader (570 nm). The applied protocol was applied within 48 and 72 hours and absorbances were obtained.

The first wells in which only the medium was left were the control group, and the viability of the cells was accepted as 100%. The percent viability rates of the cells were found with the formula given below; % viable cells = Cell absorbance of samples applied at different concentrations / Control cell absorbance × 100

Statistical Analysis

The results of the graphs related to the study were obtained with the Graphpad Prism 8 program (GraphPad Software, http://www.graphpad.com). Statistical analyzes of the MTT study were performed on a computer using SPSS 22 software. ANOVA test was performed to determine the differences between the groups studied in the MTT method. The significance level in different groups was determined according to $p < 0.05$.

Result

The IC50 value, which is the growth inhibiting effect concentration in 50% of the tumor cells, was determined using the excel program. When incubated with sheep whey Caco-2 cells, cell viability: 66.34% at 24 h; IC50 value of 8.414 µg/ml; IC50 value of 46.61% at 48 h was 5,838 µg/ml; The IC50 value of 4.61% at 72 h was found to be 4,741 µg/ml.

Sheep whey Caco-2 cell viability rate

Cell viability rates of sheep whey protein in A549 cells (control 100%) for 24h, respectively, depending on dose and time: 97.34% at 200 µg/ml; 84.89% at 400 µg/ml; 77.0% at 800 µg/ml;

72.45% at 1600 µg/ml; Viability percentages of 66.36% were found at 3200 µg/ml; for 48h: 81.78% at 200 µg/ml; 74.11% at 400 µg/ml; 54.95% at 800 µg/ml; 51.53% at 1600 µg/ml; Viability percentages of 46.61% were found at 3200 µg/ml; for 72h: 81.61% at 200 µg/ml; 80.78% at 400 µg/ml; 69.95% at 800 µg/ml; 38.19% at 1600 µg/ml; Viability percentages of 16.61% were found at 3200 µg/ml.

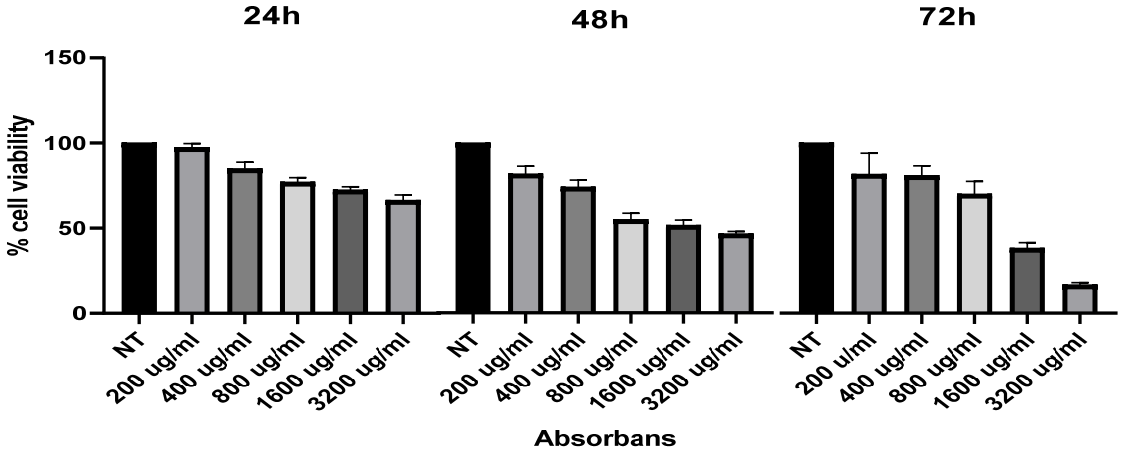

Figure 1. *Cell viability rates determined by MTT in sheep whey Caco-2 cells*

Whey protein in Caco-2 tumor cells in the control group and different concentrations for 24 hours; A significant difference was found between the control group and 400 µg/ml, 800 µg/ml, 1.600 µg/ml and 3.200 µg/ml ((p<0.001); for 48 hours; A significant difference was determined between the control group and other concentrations ((p<0.001); for 72 hours; a significant difference was determined between the control group and the control group and other concentrations ((p<0.001).

Table 1. *Sheep whey Caco-2 findings*

	24h	**48h**	**72h**
	Ort±Sd	**Ort±Sd**	**Ort±Sd**
NT	100±0,00^{ab,1}	100±0,00^{ab,1,2}	100±0,00^{ab,1,2}
200 µg/ml	95,21±2,9^{bb,2}	81,78±4,63^{bb,2}	81,61±12,46^{bb,2}
400 µg/ml	84,89±3,95^{aA,2}	74,11±4,09^{cbA,2,1}	80,78±5,76^{cb,2}
800µg/ml	77,06±2,65^{abB,2}	54,95±3,76^{abB,1,2}	69,95±7,5^{ab,2}
1600µg/ml	72,45±1,81^{ac,2}	51,52±3,10^{acC,1}	38,19±3,19^{bb,1}
3200µg/ml	66,36±3,25^{aC,1}	46,61±1,28^{abD,2}	16,61±1,28^{bbD,2}

a, b,c Absorbances in the same column with different significance levels are expressed with the same letters.

A,B,,C,DA absorbances on the same line are expressed with the same letters.

[1] The statistical difference between the absorbances shown with the same number in the same column is significant (p<0.05). [2] The statistical difference between the absorbances shown with the same number in the same column is significant (p<0.001).

Discussion

Cancer treatment; mainly applied include surgery, chemotherapy, radiotherapy and immunotherapy[10]. In general, chemotherapy, which is frequently preferred in cancer, has a negative effect on healthy cells by showing toxicity in normal cells, which can sometimes cause lifelong irreversible side effects. In addition, although the immune system of the patient receiving chemotherapy decreases, nausea, vomiting, diarrhea, hair loss, fatigue and mouth sores are seen. These disadvantages necessitate the need to develop treatment strategies and treatment supporters with minimal side effects[11]. In our work; We aimed to determine the anticancer role of colostrum components in Caco-2 cancer cells due to the many benefits they provide to human health and to reveal a new horizon in cancer patients.

In the study, lyophilized sheep whey was applied to the colorectal adenocarcinoma Caco-2 cell line for 24, 48 and 72 hours and cell viability rates were determined by MTT analysis.

Proteins in whey; α-lactalbumin, β-lactoglobulin, lysozyme, lactoferrin, serum albumin and immunoglobulins. There are study data that many of these proteins have an anticarcinogenic role[12]. In addition to whey, milk also contains casein protein[13]. In our study, we determined that sheep Caco-2 has a cytotoxic effect at the level of 84% in cancer cells.

Fakharany et al., in their study, showed that albumin protein in human, cow and camel milk inhibited CaCO2, HepG-2, PC-3 and MCF-7 cells against albumin-oleic acid complex dose-dependent tumor cells according to the MTT method[14]. In this study, we found that sheep whey colostrums showed cytotoxic activity against Caco-2 cells, depending on dose and time, according to the MTT method, Fakharany et al. supports its findings. McIntosh et al. concluded in their study that proteins in dairy products, especially whey proteins, play an important role in preventing cancer[15]. In our study, it was revealed that sheep whey proteins killed 84% of cancer cells in 72 hours at a dose of 3,200 µg/ml, which is the highest dose of CaCO 2 in cancer. Karagözlü and Bayerer reported in their study that all of the proteins found in whey show anticarcinogenic effects [16]. In our study, we concluded that whey proteins have an antiproliferative effect against Caco-2 cells.

Conclusion:

In the light of the data we obtained, it was concluded that sheep whey protein has antiproliferative activity on Caco-2 cells. It is thought that our study will contribute to the use of

sheep whey protein in cancer treatment by showing anticancer activity and will shed light on the studies to be done on this subject.

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