SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Veterınary Biochemistry

Determination of Anti Tumors Level of Caseın in Human Adenokarsinom Cell

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DOI: 10.36347/sasjm.2022.v08i08.008 | **Received:** 13.07.2022 | **Accepted:** 20.08.2022 | **Published:** 22.08.2022

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Abstract **Original Research Article**

Introduction*:** In this study, it was aimed to investigate the cytotoxicity and antitumoral activity of casein proteins obtained from bovine milk on colorectal cancer cells (Caco-2). ***Methods: Milk were fractionated as casein proteins. Antitumor activity levels of different concentrations of lyophilized proteins were measured by MTT viability test on cells and IC50 values were determined. ***Conclusion*:** Serial dilutions of casein proteins in milk, starting with 3.200 µg/ml and decreasing, were incubated with Caco-2 cells for 24, 48 and 72 hours. When incubated with bovine casein Caco-2 cells, cell viability: IC50 value found in the 24 hour run 9.172 µg/ml 69.21% at 3.200 µg/ml; IC50 at 48 hours was 8.927 cell viability; 58.5%; The IC50 value at 72 hours was 8.043 µg/ml, and cell viability was found to be 51.91%. It was concluded that bovine casein proteins have limited antiproliferative and anticarcinogenic effects on Caco-2 cancer cell lines.

Keywords: MTT Viability, Caco-2, cytotoxicity, casein.

1. INTRODUCTİON

Cancer is called malignant tumors that appear when cells in an organ or tissue divide and multiply irregularly. In general terms, cancer can be defined as the uncontrolled proliferation of cells in various parts of our body. The formation of cancerous cells from normal cells occurs by several mechanisms. As a result of mutations in oncogenes and chromosomal mutations in tumor suppressor genes, excessive proliferation of cells and inability to stop proliferation cause cancer cells [1]. Another factor in cancer is the deterioration of the genetic structure in DNA and the inability of these genes to be repaired by proteins. The proteins in question do not function due to mutation, and their genetic defects increase over time, and cell growth gets out of control, resulting in cancer cells [2].

The separation of cells from the tissue into single cells or small clusters by mechanical means followed by treatment with proteolytic enzymes and their in vitro propagation with a medium is called cell culture [3]. Bovine milk, it is rich in vitamin A and vitamin E. Among the minerals, the ratio of magnesium mineral is high. Along with the amount of globulin, the antibody concentration is also quite high. Bovine milk, the concentrations of some enzymes such as catalase, lipase and amylase are quite high, while the level of lactase enzyme is low. The color of colostrum is yellowish in the first days and is close to bitter in taste. Casein protein constitutes approximately 80% of the total protein in milk and consists of four fragments [4, 5].

Colon cancers usually start as intramucosal epithelial lesions (intramucosal carcinoma) and with the development of the tumor they reach the submucosa and become invasive cancer. Different staging methods are used in colon cancer since the spread is extremely important in terms of prognosis [6]. The vast majority of colon cancers are adenocarcinomas. Tumors other than this constitute approximately 3% of all colon cancers. These include squamous cancers (34%) and carcinoids of the colon tumors (33%) are the most common [7].

2. METHODS

The milk was delivered to the laboratory by cold chain. Bovine milk samples were fractionated into casein proteins using the isoelectric point. The lyophilization freeze-drying process of the obtained casein protein is the process of removing the water in the sample by applying a pressure of 0.070 hPa (hectopascal) at -50^{0}C. The casein protein was completely pulverized after 96 hours of lyophilization.

Preparation of cell lines: The cells kept in the cryotube were allowed to dissolve in a sterile water bath at 37^0C and were wiped with 70% alcohol and sterilized in order to prevent any contamination that may occur in the cryotubes, and then transferred to the laminar flow cabinet. Dulbecco's Modified Eagle Media (DMEM) medium containing 2.2 g/L sodium bicarbonate was used for the Caco-2 cell line.

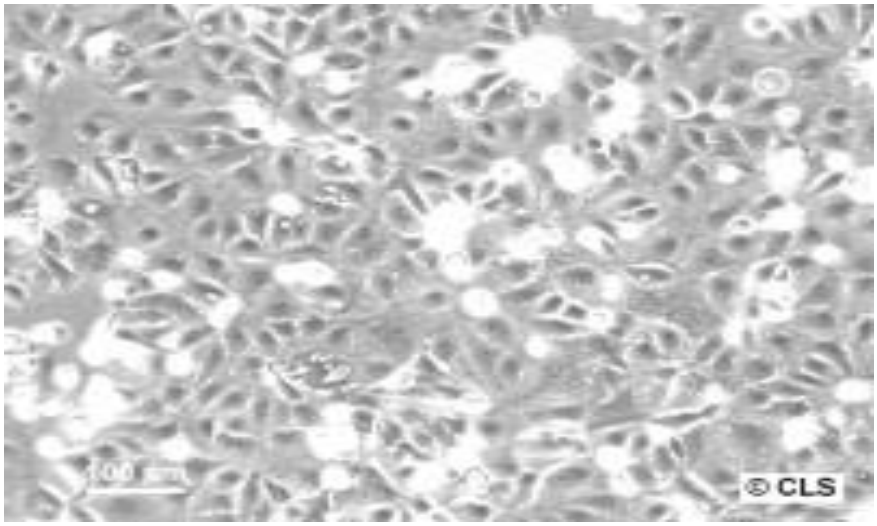

Figure 1: Caco-2 invert microscope (10 x 40)

In our study, the MTT test, which is one of the cell viability analyzes, was preferred. The basic principle of MTT analysis is based on the reduction of MTT to formason crystals through living cells, this process is applied to determine mitochondrial activity. Casein protein was weighed on a 0.064 g precision balance and dissolved in 1% DMSO (1 ml). 10 µl of casein proteins were added to 96-well plates in equal amounts to each well, with serial dilution to take at doses of 3.200 µg/ml, 1.600 µg/ml, 800 µg/ml, 400 µg/ml and 200 µg/ml. 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 oC 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 in a microplate reader (570 nm). 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 $p < 0.05$.

3. RESULTS

Viability percentages and standard deviation amounts were determined from the data obtained as a result of the MTT test study. In addition, the IC50 value and R2 (correlation coefficient) values, which are the growth inhibitory concentration in 50% of tumor cells, were determined using the excel program. The IC50 value of the bovine casein protein in Caco-2 cancer cell line in the 9.172 µg/ml 69.21% at 3.200 µg/ml; IC50 at 48 hours was 8.927 cell viability; 58.5%; The IC50 value at 72 hours was 8.043 µg/ml, and cell viability was found to be 51.91%.

Bovine casein Caco-2 cell viability rate

Cell viability study of cow casein protein in Caco-2 cells (control 100% for 24h: 96.9% at 200 µg/ml; 92.59% at 400 µg/ml; 82.92% at 800 µg/ml 81.19% at 1.600 µg/ml, 69.21% at 3.200 µg/ml viability percentages were found. For 48h: 76.33% at 200 µg/ml; 74.24% at 400 µg/ml; 69.6% at 800 µg/ml 66.72% at 1.600 µg/ml, 58.5% at 3.200 µg/ml. For 72h: 72.99% at 200 µg/ml; 68.08% at 400 µg/ml, 63.02% at 800 µg/ml, 56.72% at 1.600 µg/ml, and 51.91% at 3.200 µg/ml viability percentages were found.

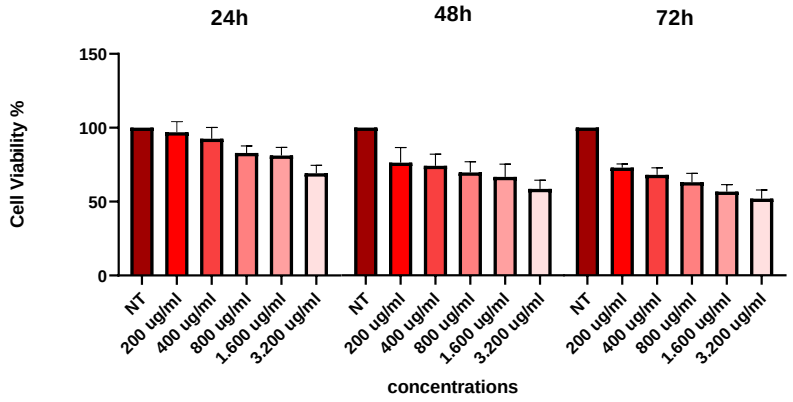

Figure 2: Cell viability rates determined by MTT in cow casein Caco-2 cells

The control group and different concentrations of bovine casein protein in Caco-2 tumor cells for 24 hours; significant difference was determined between the control group 800 µg/ml The difference between 1.600 µg/ml and 3.200 µg/ml was statistically significant ($p<0.001$); for 48 hours; there is a significant difference between the control group and other concentrations ($p<0.001$).; for 72 hours; There was no statistical difference between the control group and other concentrations ($p>0.05$); There was a significant difference between 3.200 µg/ml, 1.600 µg/ml and 400 µg/ml the control group ($p<0.001$). The statistical difference between 6400 µg/ml and other concentrations is significant ($p<0.001$).

Table 1: Bovine casein Caco-2 findings

	24h	**48h**	**72h**
	Ort±Sd	**Ort±Sd**	**Ort±Sd**
NT	100±0,00^{abcd,2}	100±0,00^{a,1}	100±0,00^{a,2}
200 µg/ml	82,10±12,93^{bcd,1}	76,33±10,26^{a,2}	73±2,43^{a,2}
400 µg/ml	78,31±12,5^{c,2}	74,25±7,85^{a,2}	78,38±8,25^{a,2}
800µg/ml	71,87±10,27^{cd,2}	69,6±8,52^{a,2}	78,81±5,39^{a,1}
1.600µg/ml	67,31±11,22^{c,2}	58,58±5,83^{a,2}	77,04±10,0^{a,2}
3.200µg/ml	58,38±9,71^{adC,1}	52,4±5,23^{a,2}	50,34±5,71^{a,2}

^{a,b,c,d,e} Absorbances in the same column that differ in significance level 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 ($p<0.001$).

4. DISCUSSION

Cancer treatment; mainly applied include surgery, chemotherapy, radiotherapy and immunotherapy [8]. 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 life-long 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 supplements with minimal side effects [9]. In our work; We aimed to determine the anticancer role of bovine milk components in Caco-2 cells due to the many benefits they provide to human health and to be able to present a new approach in cancer treatment.

It is well known that the breed, age, diet, number of births, environmental conditions and health status of the animal affect milk composition. Milk shows some differences from milk in composition and meets the nutritional requirements of the newborn at a high rate [10]. Milk secretion is regulated by both local and systemic factors. Regional and environmental factors may differ in the amount of mammary secretion depending on intramammary pressure [11]. Different studies have been conducted on casein proteins. In these studies, the effects of casein proteins gained importance. Casein protein forms the pellet part in milk, the liquid part we call supernatant is whey. The resulting casein; It contains vitamins, protein and a very small amount of milk fat. In addition, the presence of high amounts of sulfur-containing amino acids in whey protein supports its antioxidant activity [12]. While Fakharany et al. stated that bovine casein protein showed the highest cytotoxic activity, in our study the highest cytotoxic activity was found in bovine casein (tumor viability rate 51.91%). Klurfeld *et al*. suggested that the administration of casein and skim milk powder to rats with tumor cells in the colon and mammary

glands had a tumor-reducing effect [13]. In our study, the IC50 value of casein in colon cancer cell at a dose of 3.200 ug/ml in 72 hours was determined as 8.043 µg/ml and the cell viability rate was 51.91%. The obtained findings were in parallel with the findings of the study by Klurfeld *et al*., 1983 [13].

5. CONCLUSION

About 82% of mammalian bovine milk proteins are casein and the remaining 18% are whey proteins. Casein has been shown to have a high antiproliferative effect against Caco-2 cells. It has been revealed that this low effect differs depending on the dose and time. It is thought that the results obtained from the study may lead to the investigation of cellular and molecular mechanisms that may contribute to medicine in colon cancer.

Conflict of İnterest: None declared.

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