DOI: 10.37990/medr.1394291 Med Records 2024;6(1):20-6

MEDICAL RECORDS-International Medical Journal

Research Article

Can Mecsina Hemostopper, which has a Cytotoxic Effect on Mcf-7 Cells, be Considered an Anticarcinogenic Agent due to its Immunological Properties?

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Abstract

Aim: Mecsina is used as a hemostatic agent such as Ankaferd and Tranexamic acid. To struggle with breast cancer, which is a serious public health issue, new, effective, and less toxic therapeutic agents are needed. Hence, it is aimed to compare and evaluate the effects of Mecsina and Ankaferd, both of which contain natural biomolecules in their structure, and synthetic Tranexamic acid on MCF-7 cells.

Material and Method: For this study, MCF-7 immortalized cell lines were commercially purchased. The cells, 5000 cells per flask for each different dose group, were distributed to the 9 groups (mecsina 1:1, 1:2, 1:10, 1:50, 1:100, 1:200, 1:500, distilled water administered negative and control without any administration). Cytotoxicity, ELISA cytokine levels were evaluated, and flow cytometric analyzes were performed for each group using the XTT analysis method, after 24 hours of incubation.

Results: A significant difference was observed between different doses of drug administration groups of Mecsina Hemostopper hemostatic agent in MCF-7 cells ($p<0.001$). Besides, cytokine levels were found to be significantly higher than those of other possible therapeutic agents.

Conclusion: Mecsina Hemostopper has been found to have anti-tumoral activity in MCF-7 cancer cell lines by producing a hemostatic effect.

Keywords: MCF-7, cytotoxicity, flow cytometry, CD4+, CD8+, mecsina

INTRODUCTION

Breast cancer is one of the most common malignant diseases in women today, and its prevalence is increasing rapidly due to the stress of modern life (1). Since surgical resection, radiotherapy and chemotherapy are among the limited treatment options for breast cancer, new chemopreventive agents that can effectively prevent or manage breast cancer are urgently needed (2). Ankaferd Blood Stopper (ABS) is a hemostatic agent containing 5 different plant extracts. It is known to be an effective hemostopper produced for use in the fields of medicine and dentistry and routinely used in and after surgical procedures (3). Each of these plants that constitute the content of ABS has different effects on endothelium, blood cells, cell proliferation, angiogenesis and cellular mediators (4). Tranexamic acid, known to be a fibrin adhesive, is a hemostatic agent that increases vascularization and granulation tissue (5).

Today, antihemorrhagic agents actively used in the medical field can prevent bleeding by using different mechanisms of action (6). "Mecsina Hemostopper®" is created from herbal ingredients such as Mentha arvensis, Urtica angustifolia, Vitis vinifera, Hypericum perforatum, Syzygium aromaticum, Glycyrrhiza glabra extract, Alpinia officinarum. And this compound provides very important erythrocyte aggregation by forming a protein network. It has been proven with the help of electron microscopy that it binds to fibrinogen in the area exposed to the application, forming a protein network, and that erythrocytes are arranged in a roll in this network.

CITATION

Cicek M, Tumer MK, Turgut A Can Mecsina Hemostopper, which has a Cytotoxic Effect on Mcf-7 Cells, be Considered an Anticarcinogenic Agent due to its Immunological Properties?. Med Records. 2024;6(1):20-6. DOI:1037990/medr.1394291

Received: 27.11.2023 **Accepted:** 22.12.2023 **Published:** 10.01.2024

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Therefore, the hemostasis effect has also been observed (7). The main therapeutic effect of hemostatic agents such as Anakaferd, Mecsina and Tranexamic Acid is their hemostatic activity by regulating the protein network. This study aimed to compare the immunological, apoptotic, antiproliferative and cytotoxic effects of Tranexamic Acid, Ankaferd Blood Stopper, which is routinely used as a hemostatic agent during and after surgical procedures, and Mecsina Blood Stopper, a new hemostatic agent. and to examine their possible positive or negative effects on MCF-7 cancer cells.

MATERIAL AND METHOD

Cell Culture

In this study we designed, MCF-7 cells were commercially obtained from ATTC (American Type Culture Collection). DMEM and F-12 (containing 10% Fetal serum) were used as media for the cells. We provided the growth medium for the cells under environmental conditions containing 95% humidity and 5% CO_2 at 37°C. We also multiplied the cells in 25 cm^2 flasks. It was then extracted from the vial surface using 0.05% trypsin-EDTA solution. During passage, cells were transferred at a ratio of 1:2 cells per new passage. The culture medium was changed every two days after passage. After staining with Sigma brand Trypan Blue (0.05%) dye, the amount of live/dead cells was determined with the Celeromics cell counter. After reaching a sufficient number of cells, the cells were distributed into 3 groups (drug, negative treated with distilled water, and untreated control), with 5000 cells per vial for each drug. It was preserved using a DMSO-based cryopreservation protocol during the stocking process. Additionally, as is known, ethics committee approval is not required for cell culture studies. We did not receive ethics committee approval for our study.

Cell Proliferation Assay

10,000 cells were seeded in a 96-well plate for this assay. The appropriate dose was found in the cells after 24 hours of incubation by administering the drug at doses of 1:10, 1:50, 1:100, 1:200, and 1:500.

The ELISA Study

Control cells and 1:2, 1:10 dosages of drug administered MCF-7 cells were seeded in a 96-well plate at 10,000 cells.

Cell extract was obtained after 24 hours of drug treatment. In our study, we obtained the "GeneAll ProtinEx total protein extraction solution" kit by applying the manufacturer's protocol to measure antibody levels in both monolayers and supernatants. The supernatants were used for ELISA plates after measurement with Nanodrop. YEHUA Interleukin 1beta (IL-1b) (YHB1720Hu), IL-6 (YHB1747Hu) and TNF-alpha (YHB3112Hu) kits were used in the ELISA study. After the standards of the study were prepared and pipetted according to the kit protocol, the color change was measured at 450 nm wavelength after 2 hours (Eliza Device). A linear curve was created according to the Optical Density (OD) values. Concentrations were then calculated by writing the standard concentrations and OD values of the samples to the equation in the graph.

In Vitro Cytotoxicity Assays

Ankaferd, Tranexamic acid, and Mecsina Hemostopper were used in the treatment of cells. Cells and media were cultured into plates. In 6-well culture plates containing the groups we formed during the assays, we monitored the generation of 5-10 million cells. The study was started once the target number was achieved (24-48 hours). Cells were transferred to 96-well cell culture plates at 5000 per well. For 48 hours in the incubator, the cells were allowed to adhere. The XTT solution and the activation solution were mixed together. XTT was activated by mixing 25 microliters of Activation solution with 5 milliliters of XTT agent. 50uL of active XTT was taken and added to 100ul of culture medium in cell-coated wells. Cells were kept in the incubator for 24 hours. In this phase, it was analyzed at 450 nm wavelength at 2nd, 4th, 6th, 8th, 12th, and 24th hours, and the cytotoxic effect that would arise due to the dose differential between the groups was examined.

Flow Cytometric Analysis

Flow cytometric analysis was performed in Atlas Biotechnology Laboratory (Ankara, Türkiye). The intracellular cytokines TNF alpha, IL-1B, and IL-6, which are located in CD4 + and CD8 + T cells, were flow cytometrically examined separately.

Statistical Analysis

When evaluating the data, Shapiro-Wilk test is used to determine whether the variables are normally distributed, two-way analysis of variance (univariate ANOVA) is used for analyzes based on normally distributed variables and drug groups, and Tukey HSD test is used for effects in dose groups and multiple comparison tests (post-hoc). Three different tests were used: Tamhane T2 test and Dunnett test. Statistical parameters were evaluated using Mean±SD. Statistical significance was accepted as $p<0.05$. Inhibitor concentration (IC50) values were calculated based on dilution ratios. IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) and R.3.3.2 software were used to evaluate the data.

RESULTS

Cell Proliferation and Viability (%) Assessment

The dose-related cytotoxic effect of Mecsina, Ankaferd and Tranexamic Acid on MCF-7 breast cancer cell proliferation was analyzed by XTT assay (Table 1). At the end of 24 hours, cell viability rates of Ankaferd, Mecsina and Tranexamic Acid were determined at 100%, 50%, 10%, 2%, 1%, 0.05% and 0.02% concentrations. According to optical density values, the inhibition of all agents at these concentrations was compared with both the control group and each other (Table 1). It was observed that all agents showed cytotoxic effects on MCF-7 cells and there was a significant difference between the dose groups both within and between groups ($p<0.001$). All three agents showed the greatest cytotoxic effect at 100% concentration. Additionally, Mecsina Hemostopper showed a significant cytotoxic effect at concentrations of 1%, 0.05% and 0.02%.

Tranexamic Acid showed a cytotoxic effect on MCF-7 in almost all dose groups except 2% concentration (p<0.001). Almost no cytotoxic effect was observed for Ankaferd at 10%, 2% and 1% concentrations, and for Mecsina at 50% and 10% concentrations (p<0.001). Additionally, when all dose groups were evaluated, the highest IC50 concentration was observed in Ankaferd, while the lowest IC50 concentration was observed in Mecsina and Tranexamic Acid (Table 2).

Table 1. Demonstration of cytotoxicity and proliferation values of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells

		Mecsina hemostopper	Ankaferd blood stopper	Tranexamid acid	
	Concentration	Mean±SD	Mean±SD	Mean±SD	p
	Control	100.00±0.00	100.00±0.00	100.00±0.00	
	1/500	35.46±22.35**	47.57±32.05**	14.52±3.36**	0.271
	1/200	40.06±26.86**	59.97±38.15	18.72±0.04**	0.250
	1/100	38.65±27.42**b	120.97±7.29a.c	39.18±3.42**b	p<0.001*
MCF-7	1/50	58.95±1.63**b.c	116.67±1.12a.c	72.30±1.41**a.b	p<0.001*
	1/10	102.60±3.08b.c	81.19±0.94a.c	22.64±7.02**a.b	p<0.001*
	1/2	96.07±2.65b.c	3.56±0.31**a	6.20±2.65**a	p<0.001*
	1/1	6.04±1.82**b.c	0.94±0.02**a	0.73±0.25**a	p<0.001*
	p	p<0.001*	p<0.001*	p<0.001*	

Univariate;α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test;*The difference is statistically significant;**The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant

Table 2. Demonstration of IC50 values of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells

IC50	Mecsina Hemostopper	Ankaferd Blood Stopper	Tranexamid Acid
MCF-7	2.345µl	26.81µl	1.345µl

ELISA Evaluation

The immunological effects of Ankaferd, Mecsina, and Tranexamic Acid intracellular cytokines on MCF-7 breast cancer cells were evaluated both within and between groups at 1/2 and 1/10 concentrations of each cytokine (Table 3). According to the data obtained, TNF-α and IL-1B, both of which are pro-inflammatory cytokines, and the IL-6, anti-inflammatory cytokine, were found to have significant differences both for each agent separately within the group, as well as across the groups. While a significant increase was observed in TNF-α and IL-1B levels of Mecsina in 1/10 concentration compared to Ankaferd and Tranexamic Acid, IL-6 levels decreased (p<0.05). At 1/2 concentration, statistical significance was observed both between and within groups (Table 3) (p<0.05).

Table 3. Evaluation of proinflammatory and anti-inflammatory cytokines levels of Mecsina, Ankaferd and Tranexamic Acid on breast cancer cells by ELISA method

			MCF-7						
			Mecsina blood stopper ankaferd blood stopper		Tranexamid acid blood stopper		Ankaferd blood stopper		
Group	Cell	Dilution	Mean	±SD	Mean	±SD	Mean	±SD	P
IL-1B		Control	100.00	±0.00	100.00	±0.00	100.00	±0,00	
	Supernatant	1/2	123.00	±2.83**b	112.00	±1.41**a.c	129.00	±2.83**b	0.014*
		1/10	127.50	±2.12**b.c	102.00	±0.00a	106.00	±1.41a	0.001*
		P	0.002*		0.001*		0.001*		
TNF-α		Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
	Supernatant	1/2	4.00	±1.41**b.c	98.48	±0.74a.c	109.98	±1.39**a.b	p<0.001*
		1/10	125.48	±2.09**b	102.03	±0.33**a.c	121.48	±2.09**b	0.002*
		P	p<0.001*		0.011*		0.002*		
IL-6		Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
	Supernatant	1/2	81.40	±1.41**c	85.20	±1.41**c	116.00	±0.00**a.b	p<0.001*
		1/10	85.30	±2.12**c	87.70	±0.71**c	106.00	±1.41**a.b	0.002*
		P	0.002*		0.001*		0.001*		

Univariate;α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant

FLOW CYTOMETRY Results in CD4 + and CD8 + T Cells

Expressions of TNF alpha, IL-1B, and IL6, which are intracellularly released cytokines of CD4+ and CD8+ cells, were compared to the anti-hemorrhagic agents Ankaferd, Mecsina, and Tranexamic acid, as well as control groups of their various concentrations, each other, and their measurements at different times (Table 4).

Table 4: Evaluation of the levels of proinflammatory and anti-inflammatory cytokines on breast cancer cells of Mecsina. Ankaferd and Tranexamic Acid in CD4 and CD8 cells by Flowcytometry method

				Mecsina hemostopper		Ankaferd blood stopper		Tranexamid acid		
			Dilution	Mean	±SD	Mean	±SD	Mean	±SD	p
MCF-7 cell line 6 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	102.35	±0.21$^{**b.c}$	100.02	±0.01^{a}	100.65	±0.21^{a}	**0.002***
			1/10	102.70	±0.42**	102.30**	±0.28	101.60	±0.28**	0.100
			p	**0.004***		**0.001***		**0.010***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	104.00	±0.71$^{**b.c}$	100.20	±0.14^{a}	100.95	±0.21^{**a}	**0.006***
			1/10	106.55	±0.49^{**c}	103.50	±0.71^{**c}	101.35	±0.21$^{**a.b}$	**0.018***
			p	**0.005***		**0.006***		**0.009***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	360.00	±28.28$^{b.c}$	220.00	±21.21^{**a}	195.00	±7.07^{**a}	**0.008***
			1/10	829.00	±35.36$^{b.c}$	630.00	±28.28^{**a}	565.00	±21.21^{**a}	**0.006***
			p	**0.001***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	360.00	±14.14$^{**b.c}$	185.00	±±7.07^{**a}	175.00	±7.07^{**a}	**0.001***
			1/10	635.00	±21.21$^{**b.c}$	365.00	±21.21^{**a}	420.00	±28.28^{**a}	**0.003***
			p	**0.001***		**0.001***		**0.001***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	100.0	±0.01^{c}	101.35	±0.21**	103.00	±0.71^{**a}	**0.014***
			1/10	107.00	±0.28$^{**b.c}$	102.70	±0.28$^{**a.c}$	105.45	±0.49$^{**a.b}$	**0.003***
			p	**0.014***		**0.002***		**0.004***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	100.65	±0.21$^{b.c}$	103.60	±0.71^{**a}	104.75	±0.35^{**a}	**0.007***
			1/10	102.20	±0.28^{**c}	104.50	±0.71^{**c}	107.30	±0.71$^{**a.b}$	**0.008***
			p	**0.004***		**0.009***		**0.001***		
MCF-7 cell line 24 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	103.55	±0.35^{**c}	104.50	±0.71^{**c}	115.50	±1.41$^{**a.b}$	**0.002***
			1/10	124.55	±0.49$^{**b.c}$	112.00	±1.41^{**a}	116.20	±1.41^{**a}	**0.005***
			p	**0.002***		**0.002***		**0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	107.50	±1.41**	108.50	±0.71**	111.50	±2.12**	0.154
			1/10	129.50	±0.71**	112.50	±2.12**	112.80	±1.41**	0.202
			p	**0.004***		**0.005***		**0.006***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	111.00	±1.41^{**c}	119.00	±2.83^{**c}	131.00	±1.41$^{**a.b}$	**0.005***
			1/10	116.50	±2.12$^{**b.c}$	187.50	±4.24$^{**a.c}$	167.00	±2.83$^{**a.b}$	**0.001***
			p	**0.003***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	111.00	±1.41^{**c}	109.50	±0.71^{**c}	143.50	±2.12$^{**a.b}$	**p<0.001***
			1/10	165.00	±2.83^{**b}	152.50	±3.54^{**a}	147.00	±2.83**	**0.023***
			p	**0.001***		**0.001***		**p<0.001***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	114.00	±2.83**	116.50	±3.54**	114.50	±0.71**	0.650
			1/10	121.10	±1.41**	119.50	±2.83**	117.30	±2.12**	0.618
			p	**0.004***		**0.009***		**0.002***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	113.50	±2.12^{**b}	121.00	±1.41$^{**a.c}$	112.00	±1.41**b	**0.024***
			1/10	125.50	±1.41^{**b}	122.90	±2.12$^{**a.c}$	115.00	±1.41^{**b}	**0.031***
			p	**0.003***		**0.001***		**0.002***		

Univariate; α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant.

Table 4: Evaluation of the levels of proinflammatory and anti-inflammatory cytokines on breast cancer cells of Mecsina. Ankaferd and Tranexamic Acid in CD4 and CD8 cells by Flowcytometry method

				Mecsina hemostopper		Ankaferd blood stopper		Tranexamid acid		
			Dilution	Mean	±SD	Mean	±SD	Mean	±SD	p
MCF-7 cell line 72 hours	TNF-α	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	312.00	±14.14**c	300.00	±7.07**c	370.00	±14.14**a.b	**0.020***
			1/10	690.00	±28.28**	625.00	±35.36**	620.00	±28.28**	0.543
			p	**0.001***		**0.001***		**p<0.001***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	370.00	±28.28**	320.00	±14.14**	380.00	±21.21**	0.132
			1/10	830.00	±70.71**	765.00	±21.21**	794.00	±14.14**	0.064
			p	**0.003***		**0.001***		**p<0.001***		
	IL-6	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	112.00	±1.41**b.c	105.00	±0.71**a	107.30	±0.71**a	**0.013***
			1/10	119.50	±2.12**b.c	110.50	±1.41**a	111.00	±1.41**a	**0.021***
			p	**0.002***		**0.003***		**0.003***		
		CD8+	Control	100.00	±0.00	100.00	0.00	100.00	±0.00	
			1/2	104.50	±0.71**b	109.00	±1.41**a.c	104.50	±0.71**b	**0.032***
			1/10	126.30	±0.71**b	111.80	±1.41**a.c	106.20	±1.41**b	**0.031***
			p	**0.004***		**0.004***		**0.014***		
	IL-1B	CD4+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	420.00	±14.14**c	370.00	±21.21**c	213.00	±7.07**a.b	**0.002***
			1/10	720.00	±21.21**c	625.00	±28.28**c	338.00	±28.28**a.b	**0.001***
			p	**0.001***		**0.001***		**0.002***		
		CD8+	Control	100.00	±0.00	100.00	±0.00	100.00	±0.00	
			1/2	420.00	±21.21**c	283.00	±35.36**c	198.00	±14.14**a.b	**0.007***
			1/10	730.00	±28.28**b.c	725.00	±35.36**a	282.00	±28.28**a	**0.001***
			p	**0.001***		**0.001***		**0.005***		

Univariate; α:0.05;Post-hoc:Dunnett test;Tukey Test; Tamhane T2 Test; *The difference is statistically significant; **The difference compared to the control group is statistically significant; The difference according to the a Mecsina group is statistically significant; The difference compared to the b Ankaferd group is statistically significant; The difference according to c Tranexamid Acid Group is statistically significant.

An increase in the expression of all intracellular cytokines was observed in Mecsina BS-administered cell lines at 6th, 24th, and 72nd hours compared to Ankaferd BS administration in CD4 + T cells at 1/2 and 1/10 concentration ($p<0.05$). TNF-α and IL-6 levels in CD8 + T cells increased in the Mecsina-administered group relative to the other groups at 1/2 and 1/10 concentrations at the 6th hour, while IL-1B levels decreased ($p<0.005$). In comparison to the other groups, all cytokine levels at 1/10 concentration increased significantly in the Mecsina application considering the 24th hour ($p<0.05$). The levels of TNF-α and IL-6 cytokines were increased at 1/10 concentration ($p<0.05$), and the levels of IL-1B were significantly higher in the Mecsina-administered group compared to the other groups at 1/2 and 1/10 concentrations at the 72nd hour ($p<0.05$) (Table 4).

DISCUSSION

Breast cancer is the most common type of cancer in women worldwide, and its incidence increases by an average of 0.4% every year. The financial impossibilities brought about by this situation increase our search for low-cost new drugs and methods as researchers (8). The chemotherapeutic effect of existing drugs is known. However, not every patient reacts the same to these drugs, and clear answers, positive or negative, are not known about their side effects on healthy cells (9). For these reasons, it leads to the search for low-cost, fast-acting and non-toxic agents from natural bioflavonoids to treat this disease. In our study, the cytotoxicity of the anti-hemorrhagic agent Mecsina BS, which is obtained from natural compounds with limited information about its efficacy and cytotoxicity in the literature, and its effectiveness on MCF-7 cells, has been tried to be compared with another natural anti-hemorrhagic agent, Ankaferd BS, and a synthetic anti-hemorrhagic agent, Tranexamic Acid. The fact that our study is the first in this field, as determined by literature searches, increases its clinical significance. It is a condition related to tumor cell formation, decreased apoptosis and uncontrolled cell growth. For this reason, the use of cytotoxic drugs, which aim to activate apoptotic pathways and also reduce cell proliferation, is among the studies carried out for cancer treatment (10). Due to the significant side effects of chemotherapeutic drugs, researchers have recently experimented on the use of natural compounds and discovered that these compounds have high efficacy, low toxicity, and fewer side effects (11). Since the precursors of agents that induce apoptosis are

natural compounds, natural compounds are also used to induce apoptosis in human cancer cells (12,13). In this study, we aimed to evaluate the apoptotic, antiproliferative and cytotoxic effects and effectiveness of different doses of Mecsina and Ankaferd, prepared from standard plants, and Tranexamic Acid, a synthetic anti-hemorrhagic agent, on breast cancer. In this context, different doses of Mecsina, Ankaferd and Tranexamic Acid were applied to MCF-7 cells and the XTT viability test was evaluated depending on dose and time. Compared to Ankaferd, Mecsina showed a significant cytotoxic effect on MCF-7 cells at doses of 1% and lower. Tranexamic acid showed this effect at doses of 2% and lower. TNF alpha plays a key role in the activation of pro-inflammatory cytokines and leukocyte adhesion molecules. Increasing TNF-α levels increases NF-kB expression and inflammatory response (14). TNF alpha remarks the activity in the early stage of the disease. IL-1B functions as the major pro-inflammatory cytokine in the development of the Systemic Inflammatory Response Syndrome (SIRS) response (15). TNF alpha stimulates fibroblasts to release collagenase, increases vascular permeability, induces the release of cytokines such as IL-1β and IL-6, increases the production of adhesion molecules, affects the production of factors involved in osteoclast differentiation, and also increases bone resorption by showing a synergistic effect with IL-1 (16). It has been shown that, by inducing the expression of proinflammatory genes and activating stromal and immune cells, IL-1B can initiate inflammation and increase tumor activity, as well (15). In a study, increased levels of IL-1B were linked to tumor invasiveness and a poor prognosis (17). In another study, IL-1β was found to inhibit the growth of MCF-7 cells and was thought to achieve this feature together with TNF-α and IL-6 (18). IL-6 is a multifunctional cytokine that has a wide range of humoral and cellular immune effects in relation to inflammation, host defense, and tissue damage. It is released from fibroblasts in response to inflammatory stimuli such as IL-1B and TNF-α. Its biological effects include the proliferation of T cells, induction of bone resorption by synergistic effect with IL-1β (19). This is the first study to compare ABS, Mecsina, and Tranexamic Acid on MCF-7 cancer cells in the literature. In the study, changes in TNF-α, IL-1B, and IL-6 levels of possible therapeutic agents administered to MCF-7 cells and which of these agents had more anti-tumoral activity were evaluated. While the levels of TNF-a and IL-1B, which cause apoptotic cell death, were significantly higher in Mecsina application at 1/10 concentration compared to other agents, IL-6 levels were significantly lower. According to our findings, Mecsina, at a concentration of 1/10, was found to be more effective on MCF-7 cells than other therapeutic agents.

T cells are divided into three classes according to their immunological effects: helper cytotoxic T cells (Tc), regulatory T cells, and T cells (Th) (20). These cells secrete cytokines and play a role in the differentiation of activated CD4+ T cells. In contrast, Tc cells play a role in the differentiation of activated cytotoxic CD8 + T cells (21). In a study using T cell immunotherapy on MCF-7 cells, CD8 + and CD4 + T cells, which have strong antitumor effects, were selected for immunotherapy (22). It has been pointed out that subpopulations of T cells play a synergistic role in the regulation of the immune response (23). It is known that immune level adequacy is an important risk factor in the development of cancer biology and cancer prognosis, in this study, the effects of Mecsina and Ankaferd, which are bioactive compounds, and Tranexamic Acid, a synthetic molecule, on CD4 + and CD8 + T cells were investigated. It has been reported that TNF-α can induce apoptotic cell death through p38 MAPK activation. This kinase has been shown to phosphorylate Bcl-2, resulting in increased caspase-3 expression. 9 activation (24). In the study, we think that Mecsina and Ankaferd mediate the induction of apoptosis against MCF-7 cells and this may be due to an indirect effect through TNF-α. In particular, it was observed that cell viability decreased significantly. Since TNF-α activity caused by 1/10 Mecsina application increased its expression in CD8+ cytotoxic T cells. IL-6 is a molecule that induces different biological reactions depending on the function of the target cell. Additionally, it stimulates proliferation in CD8+ cells. The pleiotropic cytokine IL-1B facilitates cancer progression in various tumor types (24). Since cytokines have a significant effect on the proliferation and activation of lymphocytes, flow cytometric measurements were made of their regulatory effects on the expression of IL-1, IL-6 and TNF-α in lymphocytes activated by Mecsina, Ankaferd and Tranexamic Acid. The expression of TNF-α, IL-6, and IL-1B in CD8+ T cells in the Mecsina-treated group was found to be significantly higher than in the Ankaferd and Tranexamic Acid-treated group. It was observed that all these cytokines stimulated cytotoxic activity in MCF-7 tumor cells, especially with the application of 1/10 Mecsina.

When the results are evaluated together, it was determined that Mecsina Hemostopper has an anti-tumoral activity by producing a hemostatic effect on MCF-7 cancer cell lines. In comparison to other possible agents, Mecsina has been shown to have strong immune-enhancing, tumor-selective, and inhibitory properties. In addition, though Mecsina has been shown to be beneficial in the treatment and prevention of human cancers, more molecular studies are required to discover the molecular mechanism of this condition.

CONCLUSION

In this study, since new, effective and less toxic treatment agents are needed to combat breast cancer, which is a serious public health problem, it is known to be used as hemostatic agents in the literature, such as Mecsina, Ankaferd and Tranexamic acid. In our study, we aim to compare and evaluate the effects of Mecsina and Ankaferd, both of which contain natural biomolecules in their structure, and synthetic Tranexamic acid on MCF-7 cells. Cytotoxicity, ELISA cytokine levels were evaluated on commercially available MCF-7 cells, and

flow cytometric analyzes were performed using the XTT analysis method for each group after 24 hours of incubation. A significant difference was observed in MCF-7 cells between different dose drug application groups of Mecsina Hemostopper hemostatic agent ($p<0.001$). Additionally, it was determined that cytokine levels were significantly higher than other possible therapeutic agents. As a result, Mecsina Hemostopper was found to have anti-tumoral activity in MCF-7 cancer cell lines by producing a hemostatic substance.

Financial disclosures: *The authors declared that this study has received no financial support.*

Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *This article does not include any studies with human participants or animals by any of the authors.*

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