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# *Caspase 3, Caspase 8, Caspase 9, Granzyme B and Apaf-1 Levels in Familial Mediterranean Fever Patients Treated with Colchicine: Experimental and Theoretical Methods*

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**Abstract:** Familial Mediterranean Fever disease (FMF) is an autosomal recessive genetic disease, as well as a disease that seriously impairs the quality of life, accompanied by the appearance of symptoms such as recurrent high fever, peritonitis and acute synovitis. In FMF disease, a mutation status in the MEFV gene is encountered. The presence of faulty protein synthesis as a result of this mutation mediates the emergence of the disease. It is thought that there may be a condition associated with apoptosis in the pathogenesis of this disease. Apoptosis is a form of cell death in which energy is expended and programmed with great importance in maintaining cellular balance. In this study, the levels of Caspase 3, Caspase 8, Caspase 9, Granzyme B and Apaf-1 from apoptosis-related proteins were analyzed using the ELISA method in serum samples obtained from FMF patients treated with colchicine and a healthy control group. There was no statistically significant difference between Caspase 3 (P=0, 111), Granzyme B (P=0, 304), Apaf-1 (P=0, 097) and Caspase 8 (P=0, 245) levels in the FMF patient group compared to the control group (p >0, 05). A statistically significant difference was found in the Caspase 9 levels in the FMF patient group (P=0.001) compared to the control group (p<0.05). In this study, in which we aim to reveal the relationship between FMF and apoptosis, we can predict that drug use mediates the stabilization of apoptosis rate after an attack. However, in order for the relationship between FMF and apoptosis to be revealed more clearly, other studies using other advanced methods are also needed. However, Caspase 3 protein (PDB IDs: 2XYG), Caspase 8 protein (PDB ID: 3KIQ), Caspase 9 protein (PDB ID: 2AR9), Granzyme B protein (PDB ID: 1FQ3), and Apaf-1 protein (PDB ID: 3SDZ) were all subjected to an investigation to determine the effects of the chemical Colchicine. Research using the ADME/T method was carried out on the compounds that demonstrated the highest levels of activity.

***Keywords:*** Familial Mediterranean Fever, Colchicine, Apoptosis, Caspases, Granzyme B, Apaf-1, In silico

## 1. Introduction

FMF is an autoinflammatory disease with an autosomal recessive transition, mediating the occurrence of symptoms such as peritonitis and acute synovitis with recurrent high fever, and originating from genetic bases, in addition epigenetic factors play a role in its pathophysiology. FMF disease is mainly observed in eastern Mediterranean societies. While Turks rank first in terms of the incidence of FMF disease, the Turkish population is followed by Armenians. However, due to the reasons caused by migrations, the disease also occurs in America and European countries, albeit in small numbers [1-3].

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The process that causes FMF is associated with the incorrect synthesis of a protein called pyrin/marenostrin, which has 781 amino acids, due to a mutation in the MEFV gene on chromosome 16. Pyrin protein acts as an immune regulator in the organism. Mutations in the gene that encodes the pyrin protein cause the protein to fail to fulfill its biological function of controlling inflammation. In this case, it is not possible to control inflammation. In particular, the inflammatory response caused by various cytokines as a result of tissue damage following trauma is normally controlled by the pyrin protein. However, in FMF patients, since the pyrin protein cannot fulfill its function due to mutation, this inflammatory response cannot be controlled. In addition to being expressed in dendritic cells and granulocytes, pyrin protein can also be expressed in fibroblast cells. However, studies on pyrin protein have shown that this protein also causes caspase 1 inflammasome activation [3-5]. Pyrin protein consists of 4 domains. These are 1- N-terminal, 2- B-BOX type zinc finger, 3- Coiled Coil and 4- B30.2 C terminal. Of these domains, B30.2 C terminal is the functional group of the protein. Therefore, mutations (M694V and M694I) occurring on this domain are closely related to poor prognosis of the disease. However, the disease prognosis is also poor in the mutation called V726A [6]. In FMF patients who have the M694V mutation, there is a poor prognosis. In addition, the risk of developing amyloidosis increases in individuals with the M694V mutation [7]. In the study conducted on mutations in the MEFV gene on FMF patients in our country, it was determined that the M694I, E148Q, M694V, M680I, K695R, V726A, R761H mutations in the MEFV gene caused the disease [8]. In FMF disease, cytokines cause inflammation to be induced by increasing vascular permeability. The most functional cytokine here is IL-6 [9]. However, the increase in IFN-γ levels during the attack period of the disease may indicate that a T Helper 1-mediated immune response is needed for attacks to occur [10]. During the attack period of FMF disease, there are also increases in adhesion protein synthesis. The increase in protein synthesis such as sICAM-1 may cause immune cells to adhere to the endothelial surface and cause attacks [11]. In addition, soluble interleukin-2 receptor (sIL-2R) levels are high in FMF patient sera. This may indicate lymphocytic activation induced by the increase in soluble interleukin-2 receptor (sIL-2R) in FMF patients [12]. Finally, the dramatic changes in IL-10 levels during the course and attacks of the disease may be related to the emergence of inflammation-related symptoms of the disease [13]. However, in another study in which IL-21 and IL-23 levels were measured in the serum of patients before and after treatment of the disease, it was shown that there was no significant change in IL-21 and IL-23 levels after the beginning of the treatment [14].

The PYD region located at the N terminale has close similarities with the death domain (DD), death effector domain (DED) and CARD (caspase recruitment domain) regions that play a role in the apoptosis process. Mutations occurring in the PYD region mediate the induction of caspase 1 protein, which is responsible for apoptosis and has the ability to interact with ASC protein in particular [15]. Cytokines that play a role in the initiation of inflammation are activated by caspases for this biological process. The induction of inflammation by caspases is possible by their inclusion in an intracellular protein complex. Molecules that cause the transition from the inactive monomer state to the active dimerization state are called inflammasomes. Inflammasomes consist of caspase 1 and caspase 5 from caspase proteins, as well as the NALP-3 regions of the B30.2 region in the Pycard/ASC and MEFV gene. Caspases that have become active in this way cause the activation of proinflammatory cytokines IL-1β and IL-18. Therefore, a mutation in the structure that forms the inflammasome will directly affect caspase activation and mediate the emergence of autoinflammatory diseases [16].

Mutations in the gene encoding the pyrin protein that forms the inflammasome will disrupt the biological function of the protein, resulting in caspase 1 activation and therefore increased IL-1β synthesis, resulting in a continuous inflammatory state. IL-1 family cytokines are cut and activated via caspase-1. Although there is no similarity between the pyrin protein and IL-1 in terms of the amino acid sequence in the protein chain, caspase-1 can also cut the pyrin protein. The caspase 1 protein has the ability to cut the mutated pyrin protein at a higher rate. For this reason, it has been shown that approximately 70% of the pyrin protein

is cleaved in the peripheral blood mononuclear cells of FMF patients with pyrin gene mutations. It has been determined that after the pyrin protein is cut and divided into two, especially the N-terminal separated part of the protein mediates an increase in NF-κB activation. The cleavage of pyrin into two peptide chains accelerates the entry of p65 and NF-κB into the cell nucleus and also mediates the destruction of the kappa B inhibitor, causing NF-κB activation and thus an increase in inflammation [17].

Apoptosis, which represents the cell death program, is a physiological mechanism that controls tissue homeostasis and thus tissue formation and development in a healthy way. The apoptosis process is initiated by irreparable DNA damage, infections, cytotoxic drugs or radiotherapy, as well as by apoptosis-related proteins encoded by various genes [18]. All cells in the organism are born, differentiate, proliferate and die in a natural process. There is always a balance in this natural process. The maintenance of cellular balance can be provided by maintaining the death/proliferation balance in a healthy way through apoptosis. When the cellular balance in the tissues is disrupted, many diseases occur. While an increase in cell division rates and a decrease in apoptosis in the tissues cause cancer, conditions where there is a high rate of cell loss as a result of increased apoptosis levels are held responsible for the pathogenesis of cardiovascular diseases, especially ischemia, neurodegenerative diseases [19].

The biological events that occur during the apoptosis process are quite complex and complicated. Apoptosis is induced by energy-requiring means. When this process is induced, the nucleus condenses and breaks down. During this process, breaks also occur in the DNA and the number of breaks reaches approximately 300,000, causing cell death. Apoptosis continues with the shrinkage and termination of cellular contact in a single cell. The biggest reason for the shrinkage of the cell is the pause of the Na, K, Cl transport system. Thus, as a result of the interruption of fluid exchange, the cellular volume decreases to 1/3 and its relations with the environment are cut off with the loss of microvillius [20-22]. In addition, budding occurs in the cell membrane, and apoptotic bodies are formed from chromatin fragments surrounded by the cell cytoplasm. Then, cells exposed to this apoptotic process are recognized by neighboring cells or macrophages and phagocytosed [23]. In cells that die by apoptosis, cytokine release does not occur because membrane integrity is preserved and cell contents do not leave the cell, and therefore no inflammation occurs in the organism [24]. The recognition of apoptotic cells by phagocytizing cells is made possible by the fact that phosphotidylserine, which is located in the inner-facing region of the cell membrane, moves to the outer-facing side of the cell membrane via the aminophospholipid transferase enzyme. The receptors of the phagocytic cells, which have vitronectin and lectin properties, bind to phosphotidylserine and cause the apoptotic cell to be phagocytosed [23].

When apoptosis is induced, processes such as DNA being broken into 50-200 kb pieces by the proteolytic system, cellular proteins being destroyed, phosphatidylserine being moved from the inner side of the cell membrane to the outer side of the membrane. These processes are carried out by caspases that break peptide bonds, which are included in the protease family [25,26]. Caspases, which break the peptide bond after the aspartate residue and are normally inactive in the cell, are cysteine-protease group enzymes. Since all caspase enzymes have similar protein sequences, they show similar properties [27]. The protein chain of the caspase 3 enzyme becomes active as a result of being cut by Caspase 8 and Caspase 9 enzymes, which are the initiating enzymes of apoptosis. Active Caspase 3 mediates morphological changes in cells by cleaving target proteins at specific sites and causes apoptosis [28]. In particular, after mitochondrial damage in cells, cytochrome c is released into the cytoplasm and the complex it forms with Procaspase 9 and Apaf-1 mediates Caspase 9 activation [25,29].

T lymphocytes and natural killer cells, which are components of the immune system, synthesize the Granzyme B molecule to eliminate infected cells and cancer cells as a result of virus exposure within the organism, causing the activation of Caspase 3 via the Perforin/Granzyme pathway and thus apoptosis [26,30].

In recent years, in silico approaches have gained prominence in elucidating the molecular mechanisms underlying autoinflammatory disorders such as Familial Mediterranean Fever

(FMF), which is predominantly caused by mutations in the MEFV gene encoding the pyrin protein. Given the emerging evidence linking FMF pathogenesis to aberrant apoptotic signaling and inflammasome dynamics, theoretical computational tools offer a powerful strategy to characterize the molecular interactions of key apoptosis-related proteins [31]. In this context, the current study focuses on Caspase 3, Caspase 8, Caspase 9, Apaf-1, and Granzyme B critical regulators of intrinsic and extrinsic apoptotic pathways and their interaction with colchicine, a commonly used anti-inflammatory agent in FMF management [32]. By employing molecular docking and ADME/T analyses, this study aims to predict the binding affinities, pharmacokinetic behavior, and drug-likeness of colchicine toward these target proteins [33,34]. These theoretical insights not only contribute to a deeper understanding of apoptosis modulation in FMF but also support the identification of potential molecular targets for improved disease monitoring and therapeutic intervention.

In our study, we aimed to investigate the levels of Caspase 3, Caspase 8, Caspase 9, Apaf-1 and Granzyme B proteins associated with apoptosis in the serum of FMF patients who were diagnosed with FMF according to Tel-Hashomer criteria, treated with colchicine and carried mutations in a single chromosome in their genome, to reveal the relationship between FMF disease and apoptosis, and to reveal the contribution of these protein levels to patient follow-up in FMF disease. It was planned to investigate the levels of Caspase 3, Caspase 8, Caspase 9, Apaf-1 and Granzyme B proteins in FMF patients using colchicine because of their major roles in the apoptosis process. Caspase 3 protein (PDB IDs: 2XYG) [35], Caspase 8 protein (PDB ID: 3KIQ) [36], Caspase 9 protein (PDB ID: 2AR9) [37], Granzyme B protein (PDB ID: 1FQ3) [38], and Apaf-1 protein (PDB ID: 3SDZ) [39], were all subjected to an investigation to determine the effects of the chemical Colchicine. Research using the ADME/T method was carried out on the compounds that demonstrated the highest levels of activity.

## 2. Computational Method

### 2.1. Patient and Control Group

Thirty patients diagnosed with FMF who applied to Sivas Cumhuriyet University Faculty of Medicine Rheumatology Polyclinic were included in the study. Blood samples were taken from FMF patients after an attack and the patients were using colchicine at the time. The control group consisted of 30 healthy volunteers who did not have any chronic disease (diabetes, hypertension, cancer, FMF).

Every stage of the study was conducted in accordance with ethical principles. Before starting the research study, an ethical permission document dated 15.12.2015 and numbered 2015-12/06 was obtained from Sivas Cumhuriyet University Clinical Research Ethics Committee.

### 2.2. Collection of Blood Samples

10 ml blood samples were taken from controls and patients diagnosed with FMF. The blood samples were centrifuged at 4000 rpm for 15 minutes, and the serums obtained were aliquoted into Eppendorf tubes and stored at -80 $^{o}$C to study the relevant parameters.

### 2.3. Determination of Caspase 3, Caspase 8, Caspase 9, Granzyme B and Apaf-1 Levels

Caspase 3, Caspase 8, Caspase 9, Granzyme B and Apaf-1 protein levels were determined on an automated ELISA device (Chemwell 2902) using the ELISA kit (Elabscience, China) according to the manufacturer's instructions. The concentration of each sample was calculated from the standard curve equation created by reading the absorbance against the standard concentrations [40].

### 2.4. Theoretical calculations

Molecular docking simulations are conducted to evaluate the biological activities of compounds in relation to biological substrates. The Maestro Molecular Modeling Platform (version 13.4) created by Schrödinger [41] was used for molecular docking calculations. Calculations consist of several stages. Each step is executed individually. The protein preparation module [42] was used for protein preparation in the first stage. This module identified the active sites of the proteins. The subsequent stage involves the preparation of the analyzed compounds. Initially, the molecules are optimized using the Gaussian software program,

followed by the preparation of the LigPrep module [43] for calculations using the optimized structures. The Glide ligand docking program [44,45] was used to analyze the interactions between the compounds and the cancer protein after preparation. All computations were conducted with the OPLS4 technique. Ultimately, ADME/T study (absorption, distribution, metabolism, excretion, and toxicity) will be conducted to evaluate the pharmacological potential of the investigated compounds. The Qik-prop module [46] of the Schrödinger program was used to forecast the impacts and interactions of chemicals inside human metabolism.

### 2.5. Statistical analysis

The data of our study were loaded into the SPSS (Ver: 14.0) program and the significance test of the difference between two means and the analysis of correlation were used in the evaluation of the data and the error level was taken as 0.05.

## 3. Results and discussion

In this study, blood serum of 30 patients with definite FMF diagnosis and treated with colchicine were used. The blood serum of 30 healthy individuals without any disease were used as the control group. Patients were randomly determined without any distinction in terms of age and gender. Demographic information of the control and patient groups in the study is given in Table 1. and apoptosis-related protein levels are given in Table 2.

The control group consisted of 50% men and 50% women, while the patient group consisted of 40% men and 60% women. The gender characteristics of FMF patients and controls are given in Table 1. No statistically significant difference was found in terms of gender in both groups (P=0.436). The mean age of the patient group was 37.83 ±19.41, and the mean age of the control group was 31.13 ±10.57 (P=0.102). No statistically significant difference was found between the patient and control groups in terms of mean age. No statistically significant difference was found between the patient and control groups in terms of smoking (P=0.448).

When the levels of Caspase 3 (P=0.111), Granzyme B (P=0.304), Apaf-1 (P=0.097) and Caspase 8 (P=0.245) were examined between the patient and control groups, no statistically significant difference was found between these two groups. However, a significant difference was observed between the two groups in terms of Caspase 9 (P=0.001) levels.

**Table 1.** Evaluation of Control and Patient Groups according to Age, Gender and Smoking Habit

| | **Control** | **Patient** | ***P*** |
|---|---|---|---|
| **Age (X±S)** | 31,13 ± 10,57 | 37,83 ± 19,41 | 0,102 |
| **Gender** | | | |
| **Male N(%)** | 15 (%50) | 12 (%40) | 0,436 |
| **Female N(%)** | 15 (%50) | 18 (%60) | |
| **Smoking Use** | | | |
| **Yes N(%)** | 5 | 27 | |
| **No N(%)** | 25 | 3 | 0,448 |

**Table 2.** Evaluation of the Parameters of Control and Patient Groups

| **Parameters** | **Control** | **Patient** | ***P*** |
|---|---|---|---|
| **Apaf-1** | 1,488 ± 1,069 | 1,064 ± 0,866 | 0,097 |
| **Caspase 8** | 3,542 ± 1,100 | 3,3147 ± 1,473 | 0,245 |
| **Caspase 9** | 8,144 ± 0,878 | 7,332 ± 0,910 | 0,001 |
| **Caspase 3** | 2,044 ± 0,938 | 1,421 ± 1,886 | 0,111 |
| **Granzyme B** | 0,216 ± 0,596 | 0,101 ± 0,067 | 0,304 |

FMF is an autosomal recessive autoinflammatory disease with hereditary causes that occurs with fever and recurrent acute inflammations in serosal membranes such as peritoneum, pleura and synovium. The increased chemotactic activity of leukocyte cells in attacks seen in FMF patients causes these cells to move to the affected areas and to be found in high numbers in inflammation areas [47-49].

In order for the organism to carry out its physiological processes in a healthy way, the balance of cell proliferation and death in the tissues needs to be maintained. This balance is achieved with apoptosis, a form of programmed cell death. Many diseases can occur when this balance is disrupted. While the rapid increase in cell proliferation causes cancer, an increase in apoptosis levels can cause neurodegenerative diseases [50-52].

In our study, we aimed to determine the relationship between the disease and apoptosis and the biological processes of this possible relationship by determining the levels of apoptosis-related enzymes in the serums of FMF patients treated with colchicine. In accordance with this purpose, we determined the levels of Caspase 3, Caspase 8, Caspase 9, Granzyme B and Apaf-1 and investigated whether there were any differences between the individuals in the control group.

Caspases, which play a key role in the occurrence of apoptosis, are enzymes belonging to the cysteine-protease group. They cleave proteins from the peptide bond to which the carboxyl group of the aspartate residue is attached. They are inactive in cells. They are cut and activated as a result of various stimuli and mediate the apoptosis process by cutting specific proteins [27].

Caspase 3 causes cells to go apoptosis irreversibly by specifically cutting cellular proteins in a way that mediates apoptosis [53]. Caspase 8 enzyme activates caspase 3 by cutting the peptide bond to which the carboxyl end of the aspartate residues of caspase 3 is attached and thus mediates the irreversible occurrence of apoptosis. The activation of Caspase 9 occurs through the protein complex formed by Procaspase 9, which is inactive in the cells, and Apaf-1 and cytochrome c. When Caspase 9 is activated, the inactive Procaspase 3 protein chain is cut, causing it to become active and the process to end with apoptosis [28].

When Caspase 3 levels were compared between FMF patients and the control group in our study, a minimal decrease was observed in FMF patients, although not statistically significant. When Caspase 8 levels were compared between FMF patients and the control group, no statistical significance was observed. However, a minimal decrease was observed, as in caspase 3 levels. In our study, Caspase 9 levels were found to be statistically lower in FMF patients than in controls. The fact that blood samples were taken from FMF patients after their attacks and that they were using colchicine may have prevented possible significant changes in caspase levels. In addition, the use of colchicine in FMF patients and the fact that their blood was taken after the attack may have caused apoptosis levels to remain normal in the patients.

Apaf-1 protein, when cytochrome C is released from mitochondria to the cytoplasm as a result of apoptotic stimuli, forms a complex with cytochrome C and mediates the activation of inactive caspase 9. Thus, it causes apoptosis in the cell [54]. When we examined Apaf-1 levels in our study; we did not detect any difference between the FMF patient and control groups. Granzyme B protein is used to eliminate virus-infected cells and tumor cells by immune system cells via granulo-exocytosis. Cell death is achieved by inducing apoptosis via the Perforin/granzyme pathway, where Granzyme B protein functions [28,55]. In our study, no difference was detected between Granzyme B levels in the FMF and patient groups. The inclusion of FMF patients in the study by taking blood samples after an attack and during colchicine use may have caused the levels of Apaf-1 and Granzyme B proteins associated with apoptosis to remain normal in their blood.

Recent studies indicate that the comparison of molecular biological activities has been facilitated by substantial breakthroughs in theoretical research and technology Computations have considerably expedited and streamlined the process of identifying the most effective and successful therapies prior to experimental testing. Upon concluding the theoretical simulations, numerous parameters were discovered [56]. This technique is used to assess the biological activity of molecules by calculating the quantitative values of several parameters. The interactions between chemicals and certain proteins are the crucial component influencing the aforementioned functions. It is suggested that these interactions are quite common and ultimately prevent proteins from performing their functions properly [57]. The inhibitory process occurs via this specific mechanism. The interactions between proteins and molecules define the spectrum of energy levels that molecules exhibit. Hydrogen bonds, polar and hydrophobic interactions, π-π interactions, and halogen

interactions are the processes responsible for the interactions between molecules and proteins [58-60]. Maintaining a state of equilibrium is unattainable without molecular interactions. A comprehensive analysis of these chemical interactions has shown that molecules and proteins interact in many ways. All attributes are detailed in Tables 3, whereas Figures 1-5 through include all offered illustrations.

**Table 3**. Numerical values of the docking parameters of molecule against protein

| | Caspase 9 | Caspase 8 | Caspase 3 | Granzyme B | Apaf-1 |
|---|---|---|---|---|---|
| Docking Score | -4.34 | -3.38 | -6.14 | -3.92 | -8.29 |
| Glide ligand efficiency | -0.15 | -0.12 | -0.21 | -0.14 | -0.29 |
| Glide hbond | -0.40 | -0.16 | -0.91 | 0.00 | -0.70 |
| Glide evdw | -34.97 | -22.11 | -25.33 | -31.10 | -38.01 |
| Glide ecoul | -5.30 | -5.55 | -13.91 | -4.54 | -7.69 |
| Glide emodel | -49.61 | -31.84 | -54.54 | -41.47 | -62.10 |
| Glide energy | -40.27 | -27.66 | -39.23 | -35.65 | -45.70 |
| Glide einternal | 3.03 | 2.97 | 1.69 | 4.87 | 3.93 |
| Glide posenum | 156 | 354 | 178 | 335 | 79 |

Molecular docking simulations provide many critical statistics, with Glide Ligand Efficiency being the most significant. Furthermore, there are other supplemental qualities that are analogous to these. This numerical representation illustrates the ligand's efficacy against certain bacterial proteins. The Glide Hbond [61,62] measurement provides a quantitative assessment of the hydrogen bonds formed by interactions between molecules and proteins. The Van der Waals interaction number, often referred to as Glide Evdw [63], is an additional metric that signifies the interactions between chemicals and proteins. Furthermore, there exists a metric termed Glide Ecoul [64], offers an objective assessment of the Coulomb interactions between biological molecules and chemical entities. This quantitative statistic, referred to as the Glide Einternal, results from the integration of several components.

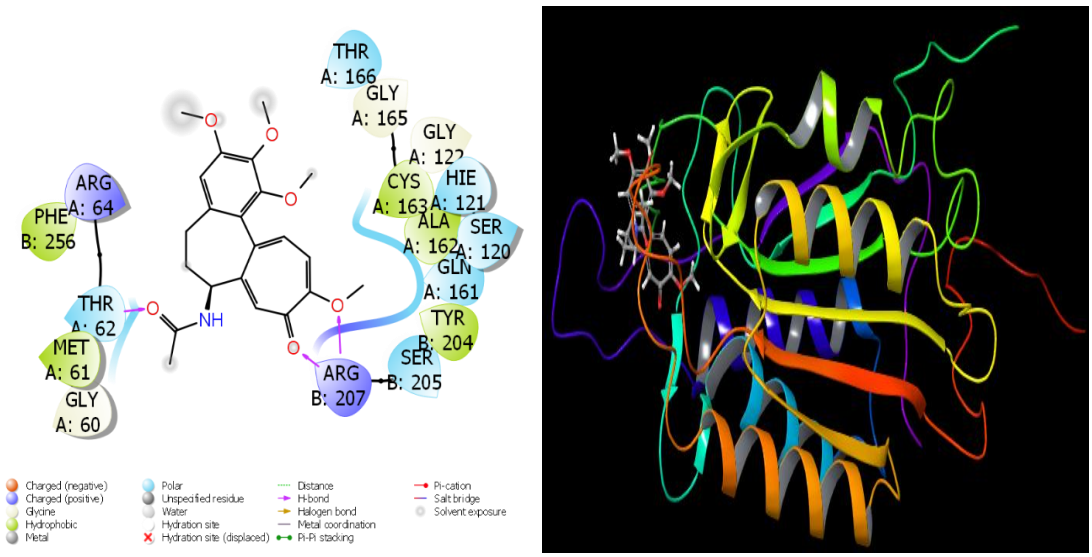


**Figure 1.** Presentation interactions of molecule with Caspase 3 (PDB ID:2XYG)

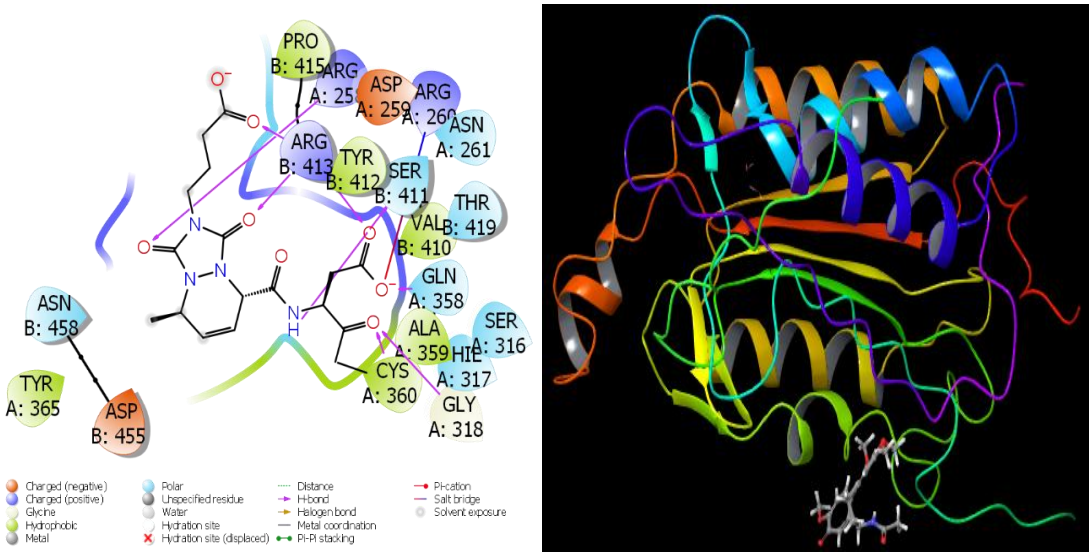


**Figure 2.** Presentation interactions of molecule with Caspase 8 (PDB ID:3KIQ)

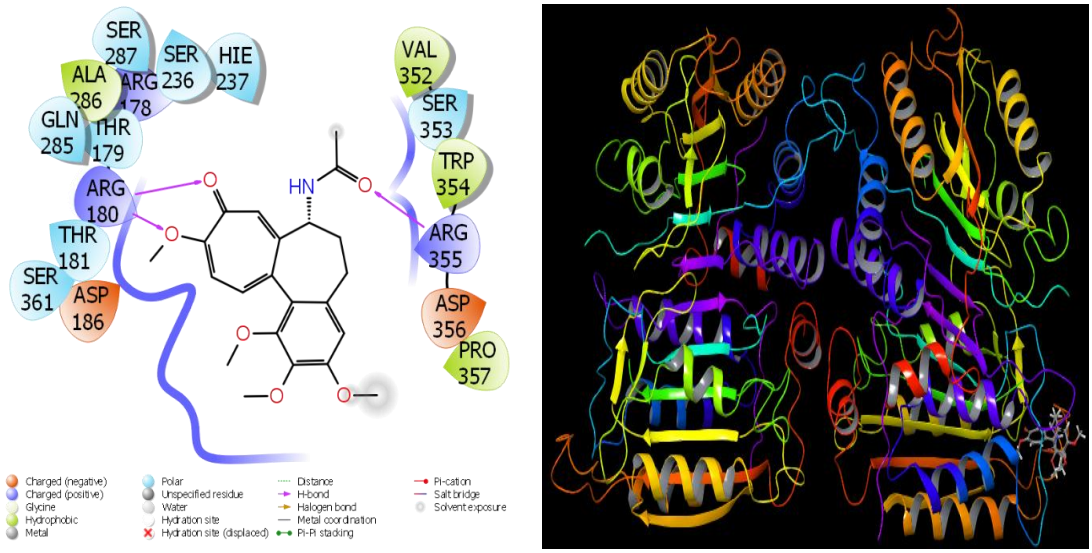


**Figure 3.** Presentation interactions of molecule with Caspase 9 (PDB ID: 2AR9)

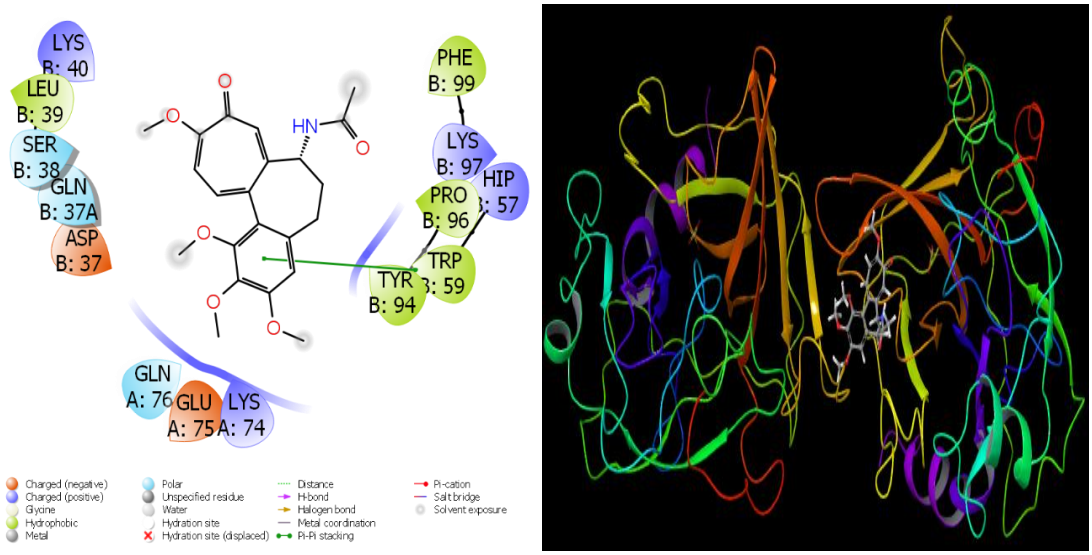


**Figure 4.** Presentation interactions of molecule with Granzyme B (PDB ID: 1FQB)

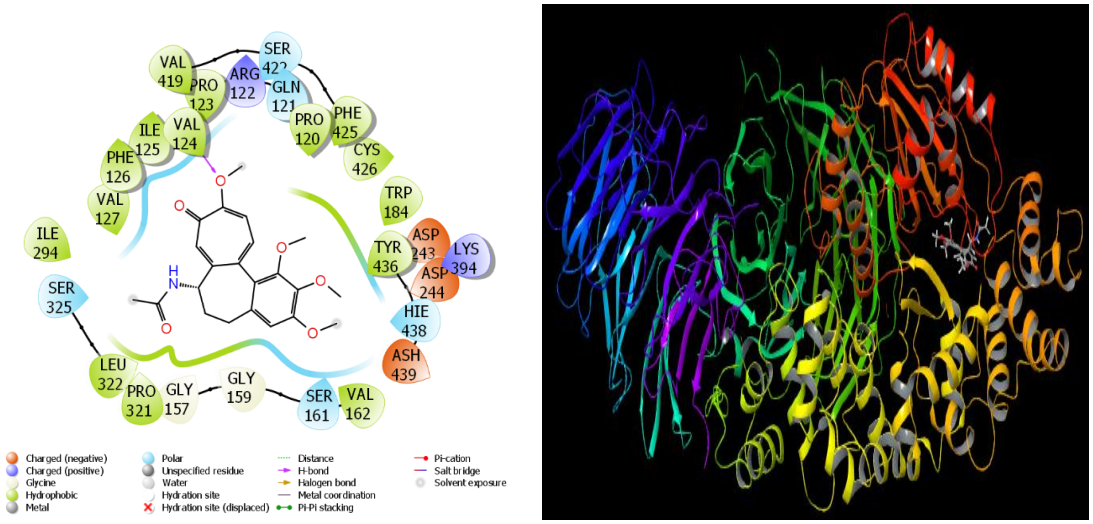


**Figure 5.** Presentation interactions of molecule with Apaf-1 (PDB ID: 3SFZ)

The pharmacokinetic parameters obtained within the scope of ADME (Absorption, Distribution, Metabolism and Elimination) analyses reveal the potential of the investigated molecule to be evaluated as a drug. In this context [65,66], some basic physicochemical and biological properties of the molecule were evaluated in Table 4.

The molecular weight (mol_MW) is 399 g/mol, which is within the reference range of 130–725 g/mol. The dipole moment was calculated as 6.5 Debye, which is acceptable in the range of 1.0–12.5. The surface area properties of the molecule were determined as SASA (solvent accessible surface area) 631 Å², FOSA (hydrophobic area) 494 Å², FISA (hydrogen bond acceptor area) 87 Å², PISA (hydrogen bond donor area) 49 Å² and WPSA (aqueous polar surface area) 0 Å². The molecular volume is 1203 Å³, which is suitable for the reference range of 500–2000 Å³ [67].

**Table 4.** ADME properties of molecule

| | **Molecule** | **Referance Range** |
|---|---|---|
| mol_MW | 399 | 130-725 |
| dipole (D) | 6.5 | 1.0-12.5 |
| SASA | 631 | 300-1000 |
| FOSA | 494 | 0-750 |
| FISA | 87 | 7-330 |
| PISA | 49 | 0-450 |
| WPSA | 0 | 0-175 |
| volume ($A^3$) | 1203 | 500-2000 |
| donorHB | 1 | 0-6 |
| acceptHB | 7.5 | 2.0-20.0 |
| glob (Sphere =1) | 0.9 | 0.75-0.95 |
| QPpolrz ($A^3$) | 39.2 | 13.0-70.0 |
| QPlogPC16 | 10.6 | 4.0-18.0 |
| QPlogPoct | 18.5 | 8.0-35.0 |
| QPlogPw | 12.4 | 4.0-45.0 |
| QPlogPo/w | 2.5 | -2.0-6.5 |
| QPlogS | -3.1 | -6.5-0.5 |
| CIQPlogS | -3.9 | -6.5-0.5 |
| QPlogHERG | -2.5 | * |
| QPPCaco (nm/sec) | 802 | ** |
| QPlogBB | -0.5 | -3.0-1.2 |
| QPPMDCK (nm/sec) | 764 | ** |

| | | |
|---|---|---|
| QPlogKp | -2.5 | Kp in cm/hr |
| IP (ev) | 9.4 | 7.9-10.5 |
| EA (eV) | 1 | -0.9-1.7 |
| #metab | 5 | 1-8 |
| QPlogKhsa | -0.1 | -1.5-1.5 |
| Human Oral Absorption | 3 | - |
| Percent Human Oral Absorption | 94 | *** |
| PSA | 94 | 7-200 |
| RuleOfFive | 0 | Maximum is 4 |
| RuleOfThree | 0 | Maximum is 3 |
| Jm | 1.1 | - |

* corcern below -5, **<25 is poor and >500 is great, *** <25% is poor and >80% is high.

The hydrogen bond donor and acceptor numbers were determined as 1 and 7.5, respectively, and these values comply with the Lipinski rules. The sphericity value (glob) is in the range of 0.90 and 0.75–0.95, supporting the compact structure of the molecule. The polarization volume (QPolrz) is 39.2 Å³ and ranges from 13–70 Å³ [68].

When the distribution coefficients are examined, the QPlogPC16 value is determined as 10.6, QPlogPoct as 18.5, QPlogPw as 12.4 and QPlogPo/w as 2.5. While these values reflect the lipophilic properties of the molecule, the logP value of 2.5 is between the ideal range of −2.0 and 6.5. The QPlogS value representing water solubility is −3.1 and the CIQPlogS value is −3.9, and these values are acceptable between −6.5 and 0.5. The QPlogHERG value indicating the HERG channel inhibition potential is above the safe limit with −2.5 (below −5 is risky) [69].

The cell permeability values, which are critical for bioavailability, were found to be quite high as QPPCaco (802 nm/s) and QPPMDCK (764 nm/s), which shows that the molecule has a good oral absorption potential. The QPlogBB value was −0.5, and its potential to cross the brain-blood barrier was at a moderate level. The QPlogKp value evaluated for skin permeability was calculated as −2.5 [70].

In terms of electronic properties, the ionization potential (IP) was found to be 9.4 eV and the electron affinity (EA) was found to be 1.0 eV. The molecule contains five metabolic sites (#metab) as a metabolism parameter. The QPlogKhsa value was −0.1, which indicates the tendency to bind to human serum albumin. Human oral absorption was evaluated as 3 out of 3, which is the highest level. In addition, the percentage of human oral absorption is 94%, and since this rate is over 80%, it shows high bioavailability [71].

The polar surface area (PSA) is 94 Å², which is within the reference range of 7–200 Å². When evaluated in terms of Lipinski's Rule of Five [72,73] and Rule of Three [74], zero deviations were observed for both rules, indicating that the molecule has drug-like properties. Finally, the Jm value was determined as 1.1.

When evaluated in general, the pharmacokinetic and physicochemical properties of this molecule show a high level of compliance with the parameters that should be considered in the drug development process and can be evaluated as a potential drug candidate.

## 4. Conclusions

We assume that the continued use of colchicine in FMF patients after an attack may be related to the normal levels of apoptosis markers in their blood. We believe that research on proteins associated with the apoptosis process will allow a better understanding of the pathogenesis of FMF disease. The theoretical analyses performed in this study provided important information about the potential interaction mechanisms of colchicine, which is widely used in the treatment of FMF, with apoptosis-related proteins. Molecular docking simulations revealed that colchicine exhibited the highest binding affinity with Apaf-1 protein (Docking Score: −8.29), followed by caspase-3 and caspase-9 proteins, respectively. Glide ligand efficiency and energy-based parameters such as Glide emodel and Glide energy also support that these interactions are stable at the molecular level. In addition, the results obtained in the ADME/T profile of colchicine were found to be quite positive in terms of pharmacokinetics. High membrane permeability (QPPCaco: 802 nm/s, QPPMDCK: 764 nm/s), optimal oral bioavailability (94%) and full compliance with Lipinski's Rule of Five and Rule of Three indicate that colchicine has systemic biological activity and has a low risk of off-target toxicity. These findings suggest that colchicine not

only interacts significantly with apoptosis-related proteins but also has drug-like properties. In conclusion, the integration of molecular docking and ADME/T analyses provides a comprehensive framework for understanding how colchicine modulates apoptosis signaling pathways in FMF. These theoretical findings strengthen the biological basis for continuing colchicine therapy in the post-relapse period and provide a rational basis for clinical applications. Furthermore, these data may shed light on new drug development studies for selectively targeting apoptosis in autoinflammatory diseases.

**Conflicts of interest**

There are no conflicts of interest in this work.

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**Ethics approval and consent to participate**

Before starting the research study, an ethical permission document dated 15.12.2015 and numbered 2015-12/06 was obtained from Sivas Cumhuriyet University Clinical Research Ethics Committee.

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