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Antiproliferative and cytotoxic activity of *Papaver somniferum* seed oil on human breast cancer cell lines

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Abstract

Background: This study aimed to determine the antiproliferative and cytotoxic activities of *Papaver somniferum* seed oil on human breast cancer (MDA-MB-231) cell lines under *in vitro* conditions.
Methods: *Papaver somniferum* seed oil extracted by cold press method were subjected to compositional analysis using GC-MS. In the study, control group, DMSO (0.1%) applied group and 5 different concentrations of *Papaver somniferum* seed oil applied group three main treatment groups were created. Cell viability was investigated by the trypan blue method. Some pharmacokinetic properties and toxicity profiles of the identified molecules were predicted using a Swiss ADME online server and ProTox-II webserver.
Results: The effect of *Papaver somniferum* seed oil on breast cancer cells increased relatively starting from the lowest concentration (87 µg/ml), and it was determined that the effect became more pronounced at concentrations above 350 µg/ml. Except Hexadecanoic acid and (E)-9- Octadecanoic acid, all other compounds were predicted to be non-hepatotoxic, non-carcinogenic, non-cytotoxic, non-immunotoxic and non-mutagenic. All compounds investigated are orally absorbable, suitable for dermal trans-distribution, have high gastrointestinal absorption, however, Hexadecanoic acid is permeable to the blood-brain barrier (BBB), while the other 4 compounds are not. The bioavailability values of all compounds investigated can be considered to be very well absorbed by the body.
Conclusion: In this study, it was determined that *P. somniferum* seed oil has antiproliferative and cytotoxic activity on breast cancer cells in vitro. *Papaver somniforum* seed oil may be a natural anticancer agent to treat breast cancer, but more studies are needed on this subject.

Keywords: breast cancer, cytotoxicity, *Papaver somniferum,* poppy, bioactivity

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Papaver somniferum tohum yağının insan meme kanseri hücre hatları üzerinde antiproliferatif ve sitotoksik aktivitesi

Özet

Amaç: Bu çalışmada, *Papaver somniferum* tohum yağının insan meme kanseri (MDA-MB-231) hücre hatları üzerindeki antiproliferatif ve sitotoksik aktivitelerinin *in vitro* koşullarda belirlenmesi amaçlanmıştır.

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Metod: Soğuk pres yöntemiyle ekstrakte edilen haşhaş tohumu yağı, GC-MS kullanılarak kompozisyon analizine tabi tutulmuştur. Çalışmada, kontrol grubu, DMSO (%0.1) uygulanan grup ve 5 farklı konsantrasyonda haşhaş tohumu yağı uygulanan grup olmak üzere üç ana tedavi grubu oluşturulmuştur. Hücre canlılığı tripan mavisi yöntemi ile araştırılmıştır. Tanımlanan moleküllerin bazı farmakokinetik özellikleri ve toksisite profilleri, bir İsviçre ADME çevrimiçi sunucusu ve ProTox-II web sunucusu kullanılarak tahmin edilmiştir.
Bulgular: *Papaver somniferum* tohumu yağının meme kanseri hücreleri üzerindeki etkisi en düşük konsantrasyondan (87 µg/ml) başlayarak nispeten artmış ve etkinin 350 µg/ml konsantrasyonundan sonra daha belirgin hale geldiği belirlenmiştir. Heksadekanoik asit ve (E)-9- Oktadekanoik asit hariç, diğer tüm bileşiklerin hepatotoksik, kanserojen, sitotoksik, immünotoksik ve mutajenik olmadığı öngörülmüştür. İncelenen tüm bileşikler oral yoldan emilebilir, dermal trans-dağılım için uygundur ve yüksek gastrointestinal emilime sahiptir; ancak Heksadekanoik asit kan-beyin bariyerine (BBB) geçirgenken, diğer 4 bileşik geçirgen değildir. İncelenen tüm bileşiklerin biyoyararlanım değerlerinin vücut tarafından çok iyi emildiği düşünülebilir.
Sonuç: Bu çalışmada, *Papaver somniferum* tohum yağının *in vitro* meme kanseri hücreleri üzerinde antiproliferatif ve sitotoksik aktiviteye sahip olduğu belirlenmiştir. *Papaver somniferum* tohum yağının meme kanserini tedavi etmek için doğal bir antikanser ajanı olabileceği, ancak bu konuda daha fazla çalışmaya ihtiyaç duyulduğu belirtilmiştir.

Anahtar kelimeler: meme kanseri, sitotoksisite, *Papaver somniferum,* haşhaş, biyoaktivite

1. Introduction

Papaver L., *Papaveraceae* Juss. It belongs to the group of annual and perennial plants in the subfamily Papaveroideae Eaton, and to the order Ranunculales Juss. The *Papaveraceae* family is a cosmopolitan family containing approximately 40 genera and 800 species, of which 170 of approximately 240 species belong to Papaveroideae. The genus Papaver consists of 134 recognized species and is distributed in the temperate and subtropical regions of the Northern Hemisphere and as far as Southern Africa. The genus is characterized as a latex-producing herbaceous annual and perennial herb. The word Papaver somniferum is used for many species belonging to the family Papaveraceae, while the word opium is mostly used for the extraction of air-dried latex from *Papaver somniferum*. All other parts of the genus Papaver, except the seeds, contain juicy and milky latex. It is secreted from cut roots, stems, twigs, leaves, flower stalks and fruits, with a latex odour, which turns milky, pale orange or pale orange-pink, brown and turns dark brown or black when dried. Latex alkaloids are used in the pharmaceutical industry and the seeds are used in the food industry [1].

Papaver somniferum is an annual herbaceous plant, 0.27–1.5 m tall. Stems are erect, with or without branches, cylindrical, glabrous and yellowish, covered with a whitish waxy layer. Leaves simple, sessile (stem leaves petiolate); Lamina lobeless or pinnate-lobed, lanceolate, lanceolate-ovate, ovate, margin irregular shallow-toothed and wavy, green above, paler below, rarely purple-green, glabrous, midrib broadly ribbed, secondary veins irregularly branched. Flowers solitary, terminal, showy, actinomorph; peduncle, 5–32 cm, glabrous; purple, red, white, colored. The fruits are capsule, first green, glabrous, yellowish, when dried, black-brown, oval, ellipsoid shaped. Seeds numerous, (593–4685 seeds per capsule), first white, creamy, brown, black-brown or black-gray [2].

Opium drugs derived from milky latex found in the unripe fruits (capsules) of the opium *Papaver somniferum* containing morphine, heroin, codeine and other alkaloids are used to make analgesics. In Central Anatolia, *Papaver somniferum* seeds are used as a stuffing in bread, bagels and pastries, ground seeds as a condiment, in muffins and pastries. *Papaver somniferum* seeds have been used since ancient times as a nutritious food and in the treatment of various ailments such as diarrhea, dysentery, cough, premature ejaculation, chronic cough, insomnia and skin conditions [3]. Its antibacterial, anti-carcinogenic and hypoglycemic potentials have also been scientifically proven [4]. Papaver somniferum seed oil is an odorless pale yellow fixed oil that can be extracted either cold or hot. It is used as food, in margarine and salad dressings and as edible. It is also used industrially as drying oil, in the manufacture of some soaps, varnishes and paints. Phytochemical studies have reported that the oil is rich in potassium, and also high in sodium, magnesium and phosphorus, and protein content. There is some information that the seeds are used as a cardiotonic, analgesic, and sedative in food, oil, and pharmaceuticals to treat inflammation. However, low levels of opium alkaloids (morphine, codeine, thebaine, noscapine and papaverine) were detected in Papaver somniferum seeds [5]. Studies with *Papaver somniferum* are mostly on the latex that the plant contains. Although there is literature information that Papaver somniferum seeds may have antitumor potential, studies on this subject are extremely few.

Bioactive molecules derived by adipose tissue are reported to be effective in regulating energy metabolism, insulin sensitivity, inflammation and vascular responses. Incompatible production of substances such as decreased adiponectin and increased tumor necrosis factor-α, interleukin-6, plasminogen activator inhibitor 1 and free fatty acids by adipose tissue contribute to the development of metabolic syndrome [6]. Treatment with fibrates, a class of lipid modifying agents, results in a significant reduction in plasma triglycerides. Generally, a moderate reduction in LDL cholesterol is associated with an increase in HDL cholesterol concentrations. Studies have shown that the effects of fibrates are mediated, at least in part, by changes in the transcription of genes encoding proteins that control lipoprotein metabolism [7]. Intracellular fatty acid binding proteins (FABPs) are abundantly expressed in almost all tissues. Fatty

acid binding proteins (FABPs) coordinate lipid trafficking and signal transduction in cells [8]. Peroxisome proliferator-activated receptors (PPAR), a subset of nuclear receptors activated by fatty acids and eicosanoids, are phosphorylated by insulin under certain conditions resulting in activation of PPARα. Gene targeting experiments in mice and genetic studies in humans have shown that these receptors play a physiological role in adipocyte function, glucose homeostasis, and lipid and lipoprotein metabolism [9]. For example, in clinical studies, PPAR-α agonists have been found to reduce the progression of atherosclerosis by modulating metabolic risk factors and by their anti-inflammatory effects at the vessel wall level [10]. It has been shown to reduce atherosclerotic plaque formation and the rate of coronary heart disease in patients with metabolic syndrome. The metabolic syndrome is characterized by a group of metabolic risk factors that include obesity, high blood pressure, dyslipidemia, insulin resistance or glucose intolerance, and a prothrombotic state [10]. It is extremely important to know the source and effect pathway of the possible bioactive effects of phenolic compounds obtained from plants. In order to determine which phenolic compounds contained in plant essential oils or fixed oils are effective, it is necessary to estimate the possible protein targets of bioactive small molecules. However, studies on possible protein targets of bioactive small molecules of *Papaver somniferum* seed oil have not been found.

In this study, it was aimed to determine possible protein targets of bioactive small molecules on human breast cancer (MDA-MB-231) cell lines of *Papaver somniferum* seed oil phenolic compounds grown in Afyonkarahisar/Türkiye *in vitro* conditions.

2. Materials and methods

2.1. Collection of plant material

In the study, whitish-yellow seeds harvested from the production areas of local producers in Afyonkarahisar/Turkey were used. After the seeds were cleaned, they were washed with distilled water, kept on filter paper at room temperature for 24 hours, then dried completely at 35°C.

2.2. Chemical composition analysis

Fixed oil was obtained by the cold pressing method. *Papaver somniferum* seed oil was rested for 48 hours and then filtered with a sterile filter and stored in dark glass bottles.

Gas Chromatography/Mass Spectrometry was used to identify the fatty acid components of *Papaver somniforum* seed fixed oil, and Gas Chromatography was used to determine their relative percentages [11]. The oil sample was derivatized with Boron Trifluoride (BF3) according to the Transmethylation Method, and the fatty acids in its composition were converted into methyl esters and the sample prepared with hexane (10% h/h) was injected into the system as 1 µL with a 40:1 split ratio.

Agilent 7890B GC System was used for Gas Chromatography (GC), and for the analyses, Agilent HP-Innowax (60m x 0.25mm inner diameter x 0.25 film thickness) column; Flame ionization Detector (FID), 250°C injection temperature; 250°C detector temperature; 60°C (10min), 4°C/min. 220°C (10 min) I°C/min 240°C (20 min), Total 100 min Carrier gas: Helium (0.7 mL/min) program temperature was used.

Agilent 7890B GC 5977B Mass Selective Detector System was used for Gas Chromatography/Mass Spectrometry (GC/MS), analyses were performed under Agilent HP-Innowax (60 m, 0.25 mm inner diameter, 0.25 pm film thickness) 250°C injection temperature, ion source temperature 230°C, 70 ev Electron energy conditions.

2.3. Anticancer activity

In the study, Dulbecco's Modified Eagle's Medium containing human breast cancer cell line (MDA-MB-231), 10% Fetal Bovine Serum and 2 mM L-glutamine; grown in 25 cm^2 flasks in the medium, in a 37 °C incubator with 5 % CO_2. Only medium was used in the control group. To dissolve the Papaver somniferum oil, Dimethyl Sulfoxide (DMSO) was used in a final concentration not exceeding 0.1 %. In the study, the control group was the group in which DMSO (0.01%) was applied and the group containing *Papaver somniferum* seed oil in 5 different concentrations (87 µg/ml, 175 µg/ml, 350 µg/ml, 700 µg/ml and 1400 µg/ml). Three main groups of operations were created. After the replicated cells were left for 24 hours, 48 hours and 72 hours incubation, trypan blue was added to examine the effects of test substances on cell viability and proliferation. After incubation, trypan blue dye was applied by trypsinification. After 24, 48, 72 hours, the amount of viable cells was counted on a neubauer slide under a light microscope, and cell viability was determined as a percentage by replacing the values in the formula [12]. Experiments were carried out 3 times and 8 repetitions. [Formula: Number of living cells/(Number of live cells+Count of dead cells)x100]

2.4. Prediction of possible protein targets of bioactive small molecules and in Silico ADMET study

SwissTargetPrediction database was used to predict possible protein targets of bioactive small molecules. Possible predictions were made based on the principle of similarity by reverse scanning. Some pharmacokinetic properties and toxicity profiles of the identified molecules were predicted using a Swiss ADME online server

(http://www.swissadme.ch, accessed on June 25, 2024), PkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction; accessed on June 28, 2024) [13]. and ProTox-II webserver (http://tox.charite.de/tox/, accessed on June 29, 2024) [14].

2.5. Statistical analysis

SPSS 21.0 package program was used for statistical evaluations. The obtained results were interpreted by applying Tamhane's T2 test according to the homogeneity of the data distribution with the analysis of variance.

3. Results

3.1. Composition of the seed oil of *Papaver somniferum*

The main component of the *Papaver somniferum* seed oil used in the study is Z,Z-9, 12-Octadecadienoic acid (73.2%) followed by (Z)-9-Octadecenoic acid (13.1%), Hexadecanoic acid (%8.8), Octadecenoic acid, (18:0) (2.3%) and (E)-9-Octadecenoic acid (1.0 %).

3.2. Anticancer Activity

The trypan blue method was used to investigate the anticancer effect of *Papaver somniferum* seed oil. In the study; Three main treatment groups were formed: the control group, the group in which DMSO (0.1%) was applied, and the group containing *Papaver somniferum* seed oil at 5 different concentrations. Normality tests (Skewness- Kurtosis test) of the obtained data set were performed and it was determined that the distribution was normal. Since it was determined that the variance was not homogeneous as a result of the analysis of variance (Levene statistics: 5.009; $p \leq 0.001$), Tamhane's T2 multiple comparison test was applied. It was determined that the average viability rate of the groups applied *Papaver somniferum* seed oil was lower than the control group and DMSO applied groups. As a result of the analysis of variance, it was determined that *Papaver somniferum* oil was effective on cell viability and this effect was statistically significant at the $p \leq 0.05$ level ($F=11.338$; Sig= 0.000). According to the results of the multiple comparison test (Tamhane's T2) performed to determine which of these three main applications the difference originated from, the difference between the control group and DMSO application was not significant, but the *Papaver somniferum* seed oil application was different from both applications and this difference was significant at the $p \leq 0.05$ level. (Figure 1). Accordingly, it was determined that the application of Papaver somniferum seed oil inhibited breast cancer cells.

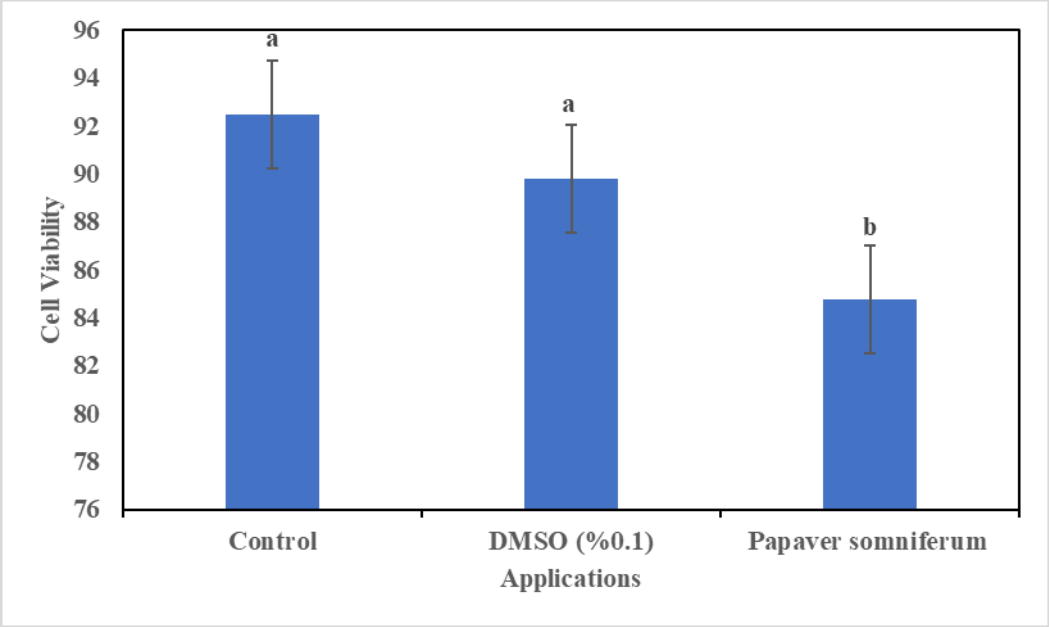

Figure 1. Total effect of *Papaver somniferum* seed oil on cell viability on breast cancer cell lines (ANOVA Tamhane's T2, $p \leq 0.05$) Abbreviations: DMSO, Dimethyl Sulfoxide

A multiple comparison test (Tamhane's T2 test) (Levene statistic: 3.287; $p \leq 0.004$) was performed to determine which oil concentration had an effect on cell viability. As a result of the analysis of variance; It was determined that the effect on cell death in breast cancer (MDA-MB-231) cell lines was statistically significant at $p \leq 0.05$ ($F= 5.664$; Sig.= 0.000), and cytotoxic activity increased depending on the increase in seed oil ratio. While the cytotoxic effect of Papaver somniferum seed oil started at 87 µg/ml and was effective on cell death, this effect became more pronounced

after 350 µg/ml. (Figure 2). Accordingly, it was determined that all concentrations of Papaver somniferum seed oil inhibited breast cancer cells.

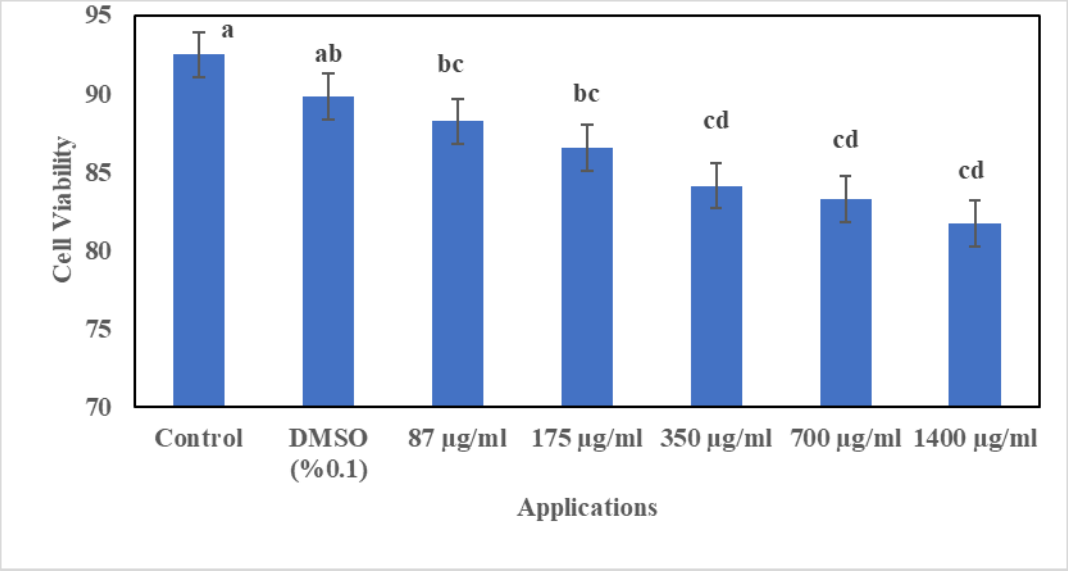

Figure 2. Cytotoxic activity of different concentrations of *Papaver somniferum* seed oil on cell viability in breast cancer cell lines (ANOVA Tamhane's T2, p≤0.05)

According to the results of the homogeneity test performed to determine the cytotoxic activity of *Papaver somniferum* seed oil on breast cancer cells, the variance of the groups was found to be homogeneous (Levene statistic=3.539; Sig.= 0.031). According to the applied Tamhane's T2 test result; It was not statistically significant between 48 and 72 hours of administration; The 24-hour application period was found to be significantly different from the other 48 and 72 hour applications at p≤0.05 (F=8.362; Sig.=0.000). Accordingly, 24, 48 and 72 hour application times of *Papaver somniferum* seed oil were found to be important in terms of cytotoxic activity on the death rates of breast cancer cells (Figure 3).

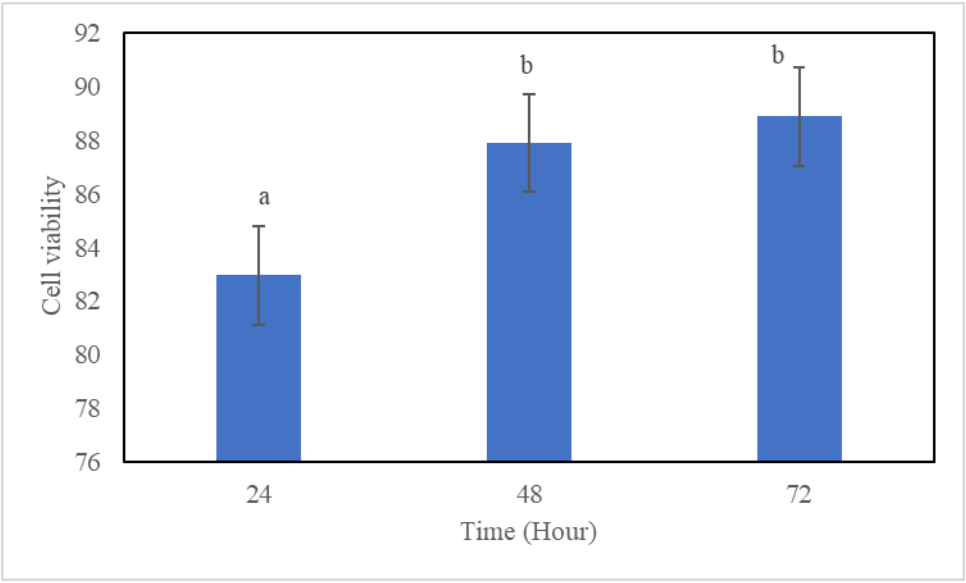

Figure 3. Cytotoxic activity of *Papaver somniferum* seed oil on breast cancer cell lines of administration (ANOVA Tamhane's T2 test, p≤0.05)

3.3. Prediction of Possible Protein Targets of Bioactive Small Molecules and in Silico ADMET Study

In studies conducted to investigate the activity level of bioactive small molecules, the bioactive target estimation of the phenolic components of *Papaver somniferum* seed oil was made separately for each compound.

In order to investigate the activity level of bioactive small molecules, bioactive target prediction of the major components found in *Papaver somniferum* seed oil was made for each compound using PubChem and SwissTargetPrediction databases. Hexadecanoic acid target prediction, one of the phenolic compounds of *Papaver somniferum* seed oil, Fatty acid binding protein adipocyte (FABP2) from Fatty acid binding protein family class at 93.5895% probability level and Peroxisome proliferator-activated receptor alpha-delta (PPARA) from Nuclear receptor class at 93.5895% probability level, indicates the PPARD) interaction.

Target prediction of (Z)-9-Octadecenoic acid, one of the phenolic compounds of *Papaver somniferum* seed oil, Peroxisome proliferator-activated receptor alpha-delta (PPARA, PPARD) from the Nuclear receptor class at a probability level of 92.9300% and Fatty from the Fatty acid binding protein family class at a probability level of 71.4850-37.0888% Acid binding protein adipocyte-epidermal (FABP1-FABP5) interaction seems possible.

One of the compounds of *Papaver somniferum* seed oil (E)-9-Octadecenoic acid target prediction at 65.7866% probability level, Nuclear receptor Peroxisome proliferator-activated receptor alpha-delta-gamma (PPARA, PPARD, PPARG) and Fatty acid at 58.3885% probability level Fatty acid binding protein from binding protein family class predicts adipocyte-epidermal-liver (FABP4, FABP5, FABP1) interaction

(Z,Z)-9, 12-Octadecadienoic acid target prediction result at 100.0000 probability level with Fatty acid binding protein adipocyte-muscle (FABP3, FABP4) interaction from Fatty acid binding protein family class at 100.0000 probability level, Nuclear indicates interaction with the Peroxisome proliferator-activated receptor gamma-alpha-delta-(PPARG, PPARA, PPARD) from the receptor class.

3.4. ADMET profiling of the identified compounds

Knowing the absorption, distribution, metabolism and toxicity (ADMET) of compounds is important for determining the potential of the molecules to be selected as drugs (Table 1).

Table 1. In silico ADMET analysis of major constituents of the seed oil of *Papaver somniferum*

Entry	**1**	**2**	**3**	**4**	**5**
Molecular weight (g/mol)	256.42	284.48	296.49	282.46	280.45
Num. heavy atoms	18,00	20	21	20,00	20
Num. arom. heavy atoms	0	0	0	0,00	0
Fraction Csp3	0.94	0.94	0.84	0,83	0.72
Num. rotatable bonds	14,00	16	16	15,00	14
Num. H-bond acceptors	2	2	2	2,00	2
Num. H-bond donors	1	1	0	1,00	1
Molar Refractivity	80.80	90.41	94.26	89,94	89.46
TPSA	37.30 Å²	37.30 Å²	26.30 Å²	37.30 Å²	37.30 Å²
Consensus Log Po/w	5,20	5,93	5,92	5,71	5,40
Lipinski rules	1	1	1	1	1
Bioavailability Score	0.85	0.85	0.55	0.85	0.85
GI absorption	High	High	High	High	High
BBB permeant	Yes	No	No	No	No
P-gp substrate	No	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log Kp (cm/s)	-2.77	-2.19	-2.82	-2.60	-2.60
PAINS	0	0	0	0	0
Brenk	0	0	1	1	1
Synthetic accessibility	2.31	2.54	3.16	3.07	3.07

1: Hexadecanoic acid; 16:0; **2**: Octadecanoic acid; (18:0); **3**: (Z)-9-Octadecenoic acid; (18:1); ω-9; **4**: (E)-9-Octadecanoic acid; (18:1); ω-9; **5**: (Z,Z)-9, 12-Octadecadienoic acid; (18:2) ; ω-6)]

Abbreviations: Fraction Csp3, Ratio of sp3 hybridized carbons over the total carbon count of the molecule; TPSA (Å2), Topological Polar surface area; Consensus Log Po/w, The partition coefficient between n-octanol and water (log Po/w) for lipophilicity; GI, gastrointestinal absorption; BBB, Blood–brain barrier; P-gp substrate, p-glycoprotein substrate; CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, Cytochromes P450 isomers; Log Kp (cm/s), skin permeability coefficient in cm/s; PAINS, Pan assay interference compounds.

According to the boiled egg results, molecules numbered 2 (Octadecanoic acid), 3 (9-Octadecenedioic acid, (9Z) and 4 (E)-9-Octadecenoic acid) remained outside the obtained model (in the white region); molecules numbered 1 (Hexadecanoic acid) and 5 (Z,Z)-9, 12-Octadecadienoic acid) remained in the boiled egg yellow region) (Figure 4).

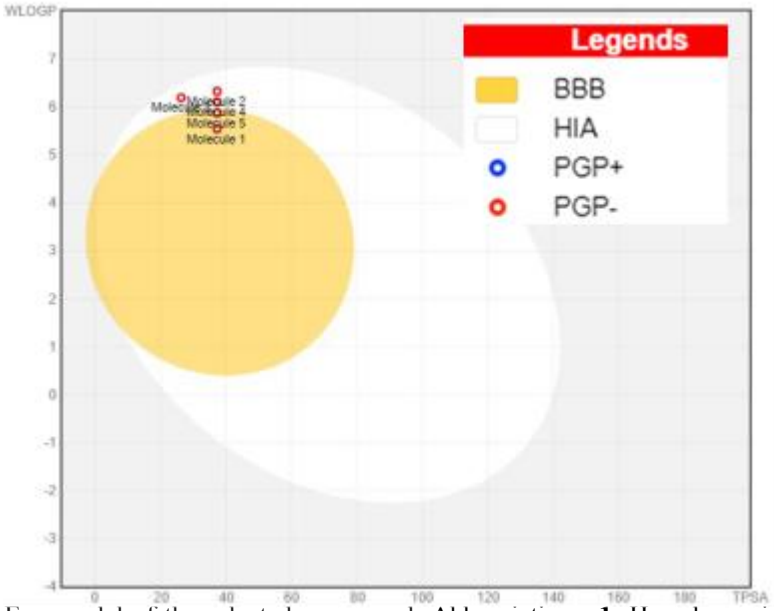

Figure 4. Boiled–Egg model of the selected compound. Abbreviations: **1**: Hexadecanoic acid; 16:0; **2**: Octadecanoic acid; (18:0); **3**: (Z)-9-Octadecenoic acid; (18:1); ω-9; **4**: (E)-9- Octadecanoic acid; (18:1); ω-9; **5**: (Z,Z)-9, 12-Octadecadienoic acid; (18:2) ; ω-6)

The investigated compounds did not inhibit the tested cytochrome P450 isoenzymes (except CYP1A2 inhibitor) (Table 1); this result may be important as O-type oxidation reactions play a fundamental role in drug biotransformation. The toxicological properties of the main components of *Papaver somniferum* seed oil were examined at the end of the study using the Swiss ADME software (Table 2). The results showed that the toxicological properties of the seed oil were within the permissible limit values.

Table 2. Toxicological properties of major constituents from the seed oil of *Papaver somniferum* using Swiss ADME software

Toxicity profile/Compound	Predicted Value				
	1	**2**	**3**	**4**	**5**
AMES toxicity	No	No	No	No	No
Max. tolerated dose (human)	-0.708	-0.791	0.040	-0.810	-0.827
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No
Oral Rat Acute Toxicity (LD50)	16.072	1.406	1.637	1.417	1.429
Oral Rat Chronic Toxicity (LOAEL)	3.181	3	3.075	3.259	3.187
Hepatotoxicity	No	No	No	No	Yes
Skin Sensitisation	Yes	Yes	Yes	Yes	Yes
T.Pyriformis toxicity	0.84	0,65	1.529	0.676	0.701
Minnow toxicity	-1.083	-1.565	-1.727	-1.438	-1.31

1: Hexadecanoic acid; 16:0; **2**: Octadecanoic acid; (18:0); **3**: (Z)-9-Octadecenoic acid; (18:1); ω-9; **4**: (E)-9- Octadecanoic acid; (18:1); ω-9; **5**: (Z,Z)-9, 12-Octadecadienoic acid; (18:2) ; ω-6)

Bioavailability polygons defined according to physicochemical parameters were created using ADMET properties of all compounds. When the bioavailability radar of the investigated compounds was examined, it showed a significantly drug-like behaviour and all compounds (except LIPO and FLEX) remained largely within the pink area of the polygon (Figure 5). The bioavailability radar of the analogues determined within the scope of the study can be considered that the pink colored region has the desired physicochemical properties for oral bioavailability.

Toxicity parameters of selected phytocompounds were estimated using web server ProTox II (Table 3). Except Hexadecanoic acid and (E)-9- Octadecanoic acid (Hepatotoxicity and Immunotoxicity), all other compounds were predicted to be non-hepatotoxic, non-carcinogenic, non-cytotoxic, non-immunotoxic and non-mutagenic. LD50 values were also calculated to ensure the safety of the studied compounds. Compounds with LD50>2000 mg/kg can be considered safe for biological application and potential drugs.

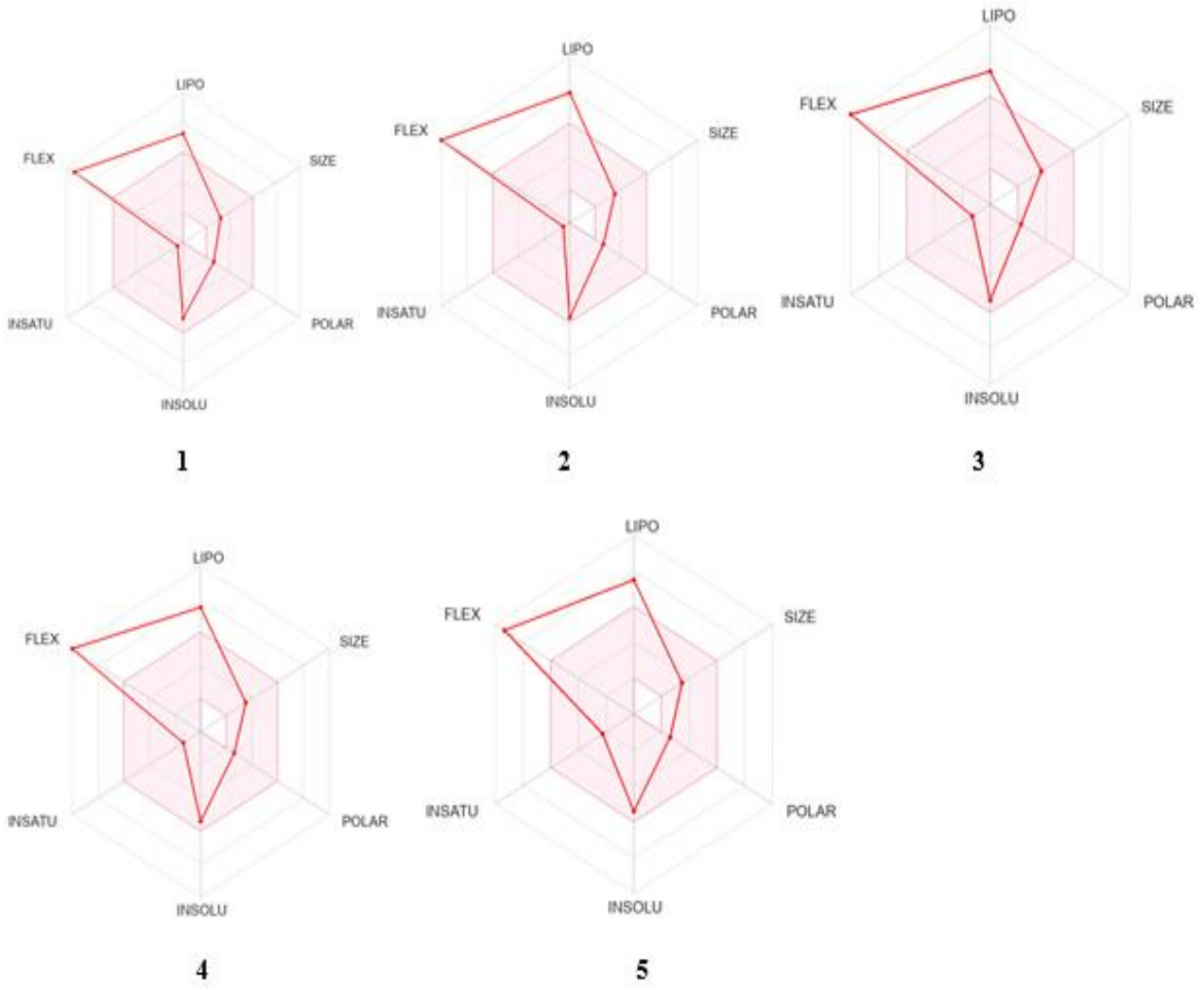

Figure 5. Bioavailability polygons of all identified compounds based on their physicochemical parameters using ADMET properties. (pink area showed the drug likeness properties of compounds). [**1**: Hexadecanoic acid; 16:0; **2**: Octadecanoic acid; (18:0); **3**: (Z)-9-Octadecenoic acid; (18:1); ω-9; **4**: (E)-9- Octadecanoic acid; (18:1); ω-9; **5**: (Z,Z)-9, 12-Octadecadienoic acid; (18:2) ; ω-6)]

Table 3. Principal toxicities of all identified compounds using ProToxII software

Compounds	Hepatotoxicity		Carcinogenicity		Immunotoxicity		Mutagenicity		Cytotoxicity		Predicted LD50	Toxicity
	Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb	(mg/kg)	Class
Hexadecanoic acid	H	0,69	NCa	0.62	I	0.96	NM	0.97	NCy	0.93	1190	IV
Octadecanoic acid	NH	0.52	NCa	0.63	NI	0.99	NM	1	NCy	0.74	900	IV
(Z)-9-Octadecenoic acid	NH	0.55	NCa	0.64	NI	0.99	NM	1	NCy	0.71	48	II
(E)-9-Octadecanoic acid	H	0.69	NCa	0.62	I	0.96	NM	0.97	NCy	0.93	1190	IV
(Z,Z)-9, 12-Octadecadienoic acid	NH	0.55	NCa	0.64	NI	0.96	NM	1	NCy	0.71	10000	VI

Abbreviations: Pr, Prediction; Pb, Probability; NH, Nonhepatotoxic; NC, Noncarcinogenic; NCy, Noncytotoxic; NI, Nonimmunotoxic; NM, Nonmutagenic; H, Hepatotoxic, I, Immunotoxic; C, Carcinogenic; Toxicity class, (Class I: fatal if swallowed (LD50 ≤ 5); Class II: fatal if swallowed (5 < LD50 ≤ 50); Class III: toxic if swallowed (50 < LD50 ≤ 300); Class IV: harmful if swallowed (300 < LD50 ≤ 2000); Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000); Class VI: non-toxic (LD50 > 5000) swallowed (300 < LD50 ≤ 2000), Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000), Class VI: non-toxic (LD50 > 5000).

4. Conclusions and discussion

Papaver somniferum is a plant native to temperate and subtropical regions of Asia and the northern hemisphere. Because of its economic importance, there are different cultivars of this plant grown in agricultural areas. The seeds are crushed (pulled) and widely used in making pancakes, poppy bread and pastries. Most of the studies on *Papaver somniferum* have focused on the white latex it secretes and the alkaloids it contains and their bioactive effects [15]. It has been reported that *Papaver somniferum* extracts, at high concentrations, destroy the cell membrane in tumor cell lines and may be an anticancer agent due to its cytostatic potential [16]. In another study, it is pointed out that *Papaver somniferum* extracts and seeds have cytotoxic, antioxidant and antitumor effects [17]. However, the effect of morphine on tumor growth is still controversial, as both growth-promoting and growth-inhibitory effects have been observed [18]. Studies on *Papaver somniferum* seed oil are more about food and nutrition. Studies on anticancer are very few.

The opium alkaloids morphine and codeine found in *Papaver somniferum* seeds were found to be the most pharmacologically active compounds, resulting in positive opiate findings in workplace-collected biological matrices and drug testing [2]. The European Food Safety Authority has set an acute reference dose of 10 µg/kg body weight as a safe level for morphine consumed through food products [5]. It is known that *Papaver somniferum* contains codeine, morphine, thebaine, stearic, palmitic, oleic, linoleic, linolenic acids and is used as a sedative, calming, sedative, pain reliever, skin and hair health enhancer, cell regenerator among the public [19]. *Papaver somniferum* seed oil is known as Oleum Papaveris drug, it is used in the preparation of some special preparations and mostly in the preparation of food as food. It is generally preferred in making poppy flatbread and poppy bread around Eskişehir [15]. In the analyzes made within the scope of this study, alkaloids were not found in the seeds. It was determined that the major components of *Papaver somniferum* seed oil are (Z,Z)-9, 12-Octadecadienoic acid (%73.2) and (Z)-9-Octadecenoic acid (%13.5), and these results are similar to Şengün et al 2020 [20]. The antimicrobial effects of *Papaver somniferum* seed oil samples on bacteria important for food microbiology were investigated and seven microorganisms (Listeria monocytogenes Scott A, Enterococcus faecalis ATCC 29212, Bacillus subtilis ATCC 6037, Staphylococcus aureus 6538P, Escherichia coli O157:H7 ATCC 43895, -4420 and Escherichia coli ATCC 1103) and that Papaver somniferum seed oils have the potential to be used as natural antioxidant and antimicrobial products [20].

Papaver somniferum seeds were found to significantly inhibit B[a]P-induced neoplasia [3]. In a study on mice, it was found that the seeds increased the activity of the carcinogenic detoxifying enzyme glutathione-S-transferase in the stomach, liver and esophagus [21]. Although it has been reported that Papaver somniferum seeds may have antitumor potential, studies on this subject are very few.

In the study, three main treatment groups were formed: the control group, the group in which DMSO (0.1%) was applied, and the group containing seed oil at 5 different concentrations. It was found that the difference between *Papaver somniferum* seed oil application and DMSO application was not statistically significant. In this study, the effect of DMSO application, which is used as the solvent of seed oil, on cell viability is not at a level to affect the results, so it can be accepted as a second control group. When all applications were compared collectively, it was determined that *Papaver somniferum* seed oil inhibited breast cancer cells (MDA-MB-231).

In the studies carried out to determine the effective oil concentration on cell viability, it was determined that the cytotoxic activity increased depending on the increase in the seed oil concentration. The cytotoxic effect of *Papaver*

somniferum seed oil increased starting from the initial concentration of 87 µg/ml, and the effect became more pronounced at concentrations above 350 µg/ml.

At the end of the studies carried out to determine the cytotoxic activity of *Papaver somniferum* seed oil on breast cancer cells (MDA-MB-231) after 24, 48 and 72 hours of incubation, the application time was found to be important. While there was no difference between the 48 and 72 hour applications, the 24-hour application was found to be lower than the other two applications. This is in contrast to the prediction that cell death will increase depending on the increase in the application time. The reason for this suggests that there may be an interaction between trypan blue and *Papaver somniferum* seed oil. In addition, it was predicted that there might be an increase in the staining rate of the dye depending on the prolongation of the time, and in such studies, application times longer than 48 hours could yield more rational results.

5 different phenolic compounds detected in *Papaver somniferum* seed oil [Hexadecanoic acid; Octadecanoic acid; (Z)-9-Octadecenoic acid; (E)-9-Octadecenoic acid; (Z,Z)-9 12-Octadecadienoic acid], the activity level of each bioactive small molecule was examined separately. All target predictions for *Papaver somniferum* seed oil components show that there may be interaction with Fatty acid binding protein adipocyte (FABP) from Fatty acid binding protein family class and Peroxisome proliferator-activated receptor (PPAR) from Nuclear receptor class at a very high probability level.

PPAR-α regulates the expression of genes involved in fatty acid β-oxidation and is a key regulator of energy homeostasis [10]. Clinical and experimental evidence suggests that PPAR activation increases the incidence of cardiovascular disease not only by correcting metabolic disorders but also through direct actions at the vessel wall level.Therefore, dysregulation of PPAR activity modulates the onset and development of metabolic disorders such as dyslipidemia, obesity, and insulin resistance that predispose to atherosclerosis [9]. Treatments that activate PPAR-α, PPAR-γ, and PPAR-δ alone or in combination have the potential to reduce cardiovascular risk despite many independent mechanisms [22]. Peroxisome proliferator-activated receptors inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. Both PPAR-α and PPAR-γ are effective in anti-inflammatory human macrophages, and activation of PPAR-a induces expression of the macrophage scavenger receptor CD36, promoting foam cell formation [23].

Fatty acid binding proteins (FABPs) are intracellular proteins expressed in almost all tissues [24]. Cellular changes in gene expression and lipid metabolism induced by FABPs are known to lead to changes in whole body energy homeostasis. Given their role in most metabolic syndrome disorders, FABPs can be considered as central regulators of lipid distribution at the cell and tissue levels, having a significant impact on systemic energy metabolism.[10] In a study in mice, adipocyte/macrophage FABPs were shown to have a potent effect on many components of the metabolic syndrome by integrating metabolic and inflammatory responses [25]. Pharmacological agents that modify FABP function can provide tissue-specific or cell-type-specific control of lipid signaling pathways, inflammatory responses, and metabolic regulation [8].

Among the compounds of *Papaver somniforum* seed oil, molecules numbered 1 (Hexadecanoic acid) and 5 [(Z,Z)-9, 12-Octadecadienoic acid] remained in the Boiled egg yellow region; other compounds remained in the white region. When the ADME properties of the compounds identified from *Papaver somniforum* seed oil were examined; since all compounds are lower than 125 Å2, they can be absorbed orally. Consensus Log Po/w values are within acceptable limits for all compounds. All of the compounds in *Papaver somniforum* seed oil exhibited high gastrointestinal absorption and skin permeability. However, only Hexadecanoic acid is permeable to the blood-brain barrier (BBB). In terms of bioavailability values, all of the compounds can be considered to be very well absorbed by the body. It was observed that the compounds examined did not inhibit the tested cytochrome P450 isoenzymes (except for CYP1A2 inhibitor). When the bioavailability radar of the investigated compounds was examined, it remained largely within the pink area of the polygon (except LIPO and FLEX); accordingly, *Papaver somniferum* seed oil can be considered to have the desired physicochemical properties for oral bioavailability. When the toxicity parameters of the selected phytocompounds were examined, except for Hexadecanoic acid and (E)-9- Octadecanoic acid (Hepatotoxicity and Immunotoxicity), all other compounds were predicted not to be hepatotoxic, carcinogenic, cytotoxic, immunotoxic and mutagenic. According to the LD50 values of the investigated compounds, *Papaver somniferum* seed oil can be considered safe for biological application and as a potential drug.

In conclusion, in this study, it was determined that *Papaver somniferum* seed oil phenolic compounds inhibited breast cancer cells (MDA-MB-231) in-vitro conditions. In addition, the bioactive effect might have been caused by the interaction of Fatty acid binding protein adipocyte (FABP) from the Fatty acid binding protein family and Peroxisome proliferator-activated receptor (PPAR) from the Nuclear receptor family. The main challenge is to develop new compounds that maximize potential benefits while minimizing the undesirable effects of compounds found in seed oil. The results of this study discuss that *Papaver somniforum* seed oil may be a natural anticancer agent to treat breast cancer and may be safe to use in the food industry. With this feature, the study may contribute to the development of natural anticancer agents. More work is needed on this subject.

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