RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2025; 39 (1): 15 - 21
http://www.fusabil.org

Effect of *Nigella sativa* Oil on Pyruvate Kinase Activity in Kidney Tissue in Doxorubicin-Treated Rats: ADME Predictions and Molecular Docking Studies

Harun USLU [1, a]
Emre KAYA [2, b]
Seval YILMAZ [2, c]

[1] Fırat University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Elazığ, TÜRKİYE

[2] Fırat University, Faculty of Veterinary Medicine, Department of Biochemistry, Elazığ, TÜRKİYE

[a] ORCİD: 0000-0001-8827-8557
[b] ORCİD: 0000-0002-7445-3091
[c] ORCİD: 0000-0002-2040-9247

This study aimed to investigate the effect of *Nigella sativa* oil (NSO) on pyruvate kinase (PK) after Doxorubicin (DOX) application in kidney tissue. When the DOX group was compared with the control group, a decrease in PK activity was found, and a significant difference was found in PK activity. While no statistically significant difference was found between the group administered only NSO and the control group, it was determined that PK activity increased statistically significantly in the group administered NSO together with DOX compared to the DOX group, and the values approached control group values. According to recent studies, *in silico* molecular docking studies and ADME predictions were performed by selecting the compounds in the highest amounts in the fixed oil and essential oil obtained from NSO. Using molecular docking, we looked into how NSO affected PK. Some selected NSO components were shown to interact similarly with the cocrystal ligands of PK. Molecular docking studies showed that Palmitic acid (c) and Thymoquinone (e) were one of the selected compounds with the best pose on the PK, for fixed and essential oils respectively. It was estimated that NSO fixed oil components would inhibit at PK nanomolar level (4.57 nm-17.72 nm) and NSO essential oil components would inhibit at PK micromolar level (52.05 µM-255.92 µM). Considering ADME predictions Oleic acid (b), Palmitic acid (c), p-Cymene (d), Thymoquinone (e), and Carvacrol (f) had good pharmacokinetic profiles, but Linoleic acid (a) did not find drug-likeness properties.

Key Words: *ADME, molecular docking, nigella sativa oil, doxorubicin, pyruvate kinase*

Doksorubisin ile Tedavi Edilen Sıçanlarda Böbrek Dokusunda Pirüvat Kinaz Aktivitesi Üzerine *Nigella sativa* Yağının Etkisi: ADME Tahminleri ve Moleküler Docking Çalışmaları

Bu çalışmada, *Nigella sativa* yağının (NSO) böbrek dokusunda Doksorubisin (DOX) uygulamasından sonra pirüvat kinaz (PK) üzerine etkisinin araştırılması amaçlanmıştır. DOX grubu, kontrol grubu ile karşılaştırıldığında PK aktivitesinde azalma ve anlamlı bir fark bulunmuştur. Sadece NSO uygulanan grup ile kontrol grubu arasında istatistiki fark saptanmamışken DOX ile beraber NSO uygulanan grupta, DOX grubuna göre istatistiki olarak anlamlı düzeyde PK aktivitesinin arttığı belirlenmiş olup değerlerin kontrol grubu değerlerine yaklaştığı saptanmıştır. Son çalışmalara göre, NSO'dan elde edilen sabit yağ ve uçucu yağda en yüksek miktarda bulunan bileşiklerden bazıları seçilerek *in silico* moleküler yerleştirme çalışmaları ve ADME tahminleri yapılmıştır. Moleküler docking kullanılarak, NSO'nun PK'yı nasıl etkilediğine bakılmıştır. Seçilen bazı NSO bileşenlerinin, PK'nin kokristal ligandlarıyla benzer şekilde etkileşime girdiği görülmüştür. Moleküler docking çalışmaları, Palmitik asit (c) ve Timokinon'un (e), sabit yağ ve uçucu yağ için sırasıyla PK üzerinde en iyi poza sahip seçilen bileşiklerden biri olduğunu göstermiştir. NSO sabit yağ bileşenlerinin PK'yı nanomolar düzeyde (4.57 nm-17.72 nm), NSO esansiyel yağ bileşenlerinin ise PK'yı mikromolar düzeyde (52.05 µM -255.92 µM) inhibe edeceği tahmin edilmiştir. ADME öngörüleri göz önüne alındığında Oleik asit (b), Palmitik asit (c), p-Simen (d), Timokinon (e), ve Karvakrol'ün (f) iyi farmakokinetik profil gösterdiği, ancak Linoleik asit'in (a) ilaç benzerlik özelliği göstermediği bulundu.

Anahtar Kelimeler: *ADME, moleküler docking, çörek otu yağı, doksorubisin, pirüvat kinaz*

Received : 15.10.2024
Accepted : 14.01.2025

Correspondence

Harun USLU
Fırat University,
Faculty of Pharmacy,
Department of
Pharmaceutical Chemistry
Elazığ – TÜRKİYE

huslu@firat.edu.tr

Introduction

Black cumin (*Nigella sativa*) is an annual flowering plant from the Ranunculaceae family. Its seeds have been used as food for centuries worldwide and are a nutritious source, and its oil is rich in phytochemicals (1). Black cumin seeds have a slightly bitter taste similar to thyme and are used in salads or to add a bitter flavor to vegetable dishes. Many species of black cumin seeds can be grown as ornamental plants and are dried and used in flowers for decoration. In his books, Ibn-Sina mentions that black cumin give energy to the body, stimulate it, relieve fatigue, and boost morale (2). This content is protective against toxicity in different tissues (kidney, stomach, heart, liver) in many studies (3-6). Since ancient times, black cumin (*Nigella sativa*) seeds and oil have been used in the Middle East, India, and Africa to treat diseases such as headache, cough, eczema, and fever (7). It seems that thymoquinone, especially found in its oil and seed, causes these pharmacological effects. Another very important effect of black cumin, which can be used in various treatments, is its effect on cancerous cells. It has been observed that both the oil and other active components

(thymoquinone) have antimicrobial and anticancer effects by acting on a wide variety of microbes and uncontrollably proliferating cancer cells (8).

Pyruvate kinase is an enzyme that plays an important role in the regulation of cell metabolism in ATP conversion and glycolysis (9). Muscle isoform 2, namely PKM2, one of the isoforms of pyruvate kinase, plays a critical role in the growth of cancer as well as regulation. Although studies conducted so far have said that only PKM2 is involved in cancer growth, new studies show that the other isoform, PKM1, also plays a role in this (10). It has been shown that the regulation of PKM2, which is especially abundant in kidney tissues, positively affects the treatment of kidney diseases (11). Cancer, the deadliest type of disease in the world, causes the death of approximately 10 million people annually. Statistically, it can be said that 1 in 6 of all deaths are due to cancer (12).

Doxorubicin (DOX), an antibiotic agent obtained from the Streptomyces peucetius bacterium and used in cancer therapy since the 1970s, has also been described as an anthracycline group chemotherapeutic. It is actively used in a wide variety of cancer types such as breast cancer, bone sarcomas, and thyroid cancer (13). Although it has a very wide range of effects and mechanisms, dilated cardiomyopathy, which means irreversible weakening of the heart muscle and decreased contractile function, has been observed in patients receiving long-term treatment with DOX (14).

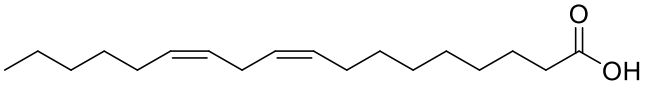
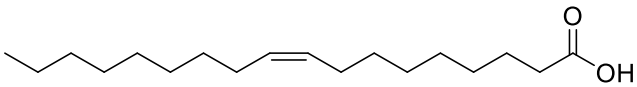
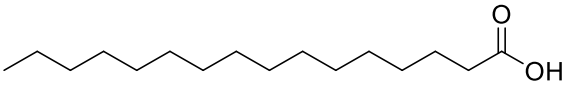
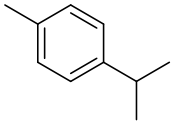
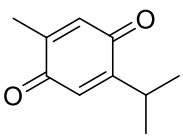
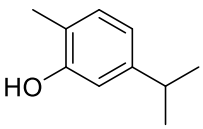
Due to its wide spectrum of biological activity, Nigella sativa oil (NSO) has recently been increasingly used to prevent health-related problems. It was aimed to investigate the effect of NSO on PK activity in doxorubicin-induced kidney damage. This evaluation included the biochemical study that uses rats to investigate NSO prevention mechanisms against kidney tissue toxicity induced by DOX. In this study, we revealed the molecular docking and ADME prediction of some components of NSO (Table 1). In this context, the *in vivo* biochemical results and *in silico* molecular docking results, and ADME predictions were compared and evaluated. In addition, it is desired to contribute to the development of NSO prevention of DOX-induced kidney tissue damage.

Materials and Methods

Research and Publication Ethics: Fırat University's local animal experiment ethics committee approved this research with Protocol Number 2012/03-43.

***In vivo* Studies:** In the study, 28 three-month-old male Wistar-Albino (Rattus norvegicus) rats with an average weight of 275 g were obtained from Fırat University Laboratory Animal Breeding Unit and used. Rats were kept in air-conditioned rooms under standard conditions, at a constant temperature of 25±2ºC, 60-65% humidity, 12 hours light/12 hours dark cycle. They were fed with chow in pellet form and tap water as *ad libitum* during the experimental procedures (15). Experimental practices on rats were performed in the Fırat University Experimental Research Center.

Table 1. Chemical Structure of some components of NSO

Code	Compounds	Chemical Structure
a	Linoleic acid	
b	Oleic acid	
c	Palmitic acid	
d	*p*-Cymene	
e	Thymoquinone	
f	Carvacrol	

The oil of black cumin seeds was bought from Gebece Bitkisel (Antalya, Türkiye). In the study, the rats were divided into 4 groups, each including 7 rats: the first group: control group, the second group: the group that received *Nigella sativa* oil (NSO) 2 mL/kg/day by gavage for 7 days, third group: the group that received single dose 20 mg/kg body weight Doxorubicin (DOX) intraperitoneally, and fourth group: the group that received NSO (2 mL/kg/day by gavage, 7 days) + DOX (20 mg/kg body weight, intraperitoneal single dose). In this group, NSO application was started 2 days before DOX administration and continued for 7 days. DOX was bought from Fresenius Kabi Oncology Ltd. (Solan, India). The amount of DOX used in the study was determined based on the previous studies (1, 16). Rats in the DOX group were killed by decapitation 5 days after the DOX application, and rats in the control and NSO groups were killed 7 days after the beginning of the experiment. In the NSO+DOX group, NSO pretreatment was applied for 2 days, then DOX was applied, and rats in the group were killed by decapitation 5 days after DOX application. The activities of the PK enzyme were determined spectrophotometrically (Thermo Scientific, Genesys 10S, USA) in the kidney tissue. At the end of the practice, rats were sacrificed and kidney tissue samples were collected. Tissue samples were stored in a deep freezer at -80°C until biochemical analysis. Kidney tissue samples were washed with physiological saline solution, diluted with distilled water according to the weight-volume ratio (1:10), and homogenized using a Potter-elvehjem homogenizer (CAT R50D, Germany). For PK activity analysis, homogenates were centrifuged at 13,500 rpm for 55 minutes at +4°C (NUVE NF800R, Türkiye). PK activity was measured spectrophotometrically based on measuring the rate of decreasing absorbance of NADH at 340 nm by the method modified by Beutler *et al*. (17). The method of Lowry *et al*. (18) was used to determine the protein concentration in the tissue homogenate, while protein levels were used to calculate the specific activity of the enzyme (19).

Molecular Docking Studies: According to the study conducted by Sultan et al., the compounds found in the highest amounts of fixed oil and essential oil obtained from black cumin seeds are shown in Table 2 (20). Other components are not shown in this table and, therefore, do not add up to 100%. According to these results, *in silico* molecular docking studies and ADME predictions were carried out on the compounds in Table 2.

Table 2. Some of the components were found to be in high amounts by mass in the fixed oil and essential oil of black cumin seed (20)

	Fixed oil	%		Essential oil	%
a	Linoleic acid	57.38±1.53	d	*p*-cymene	32.02±1.01
b	Oleic acid	19.65±0.61	e	Thymoquinon	23.25±1.03
c	Palmitic acid	12.07±0.87	f	Carvacrol	10.38±0.30

The chemical structures of all selected components (a-f) of *Nigella sativa* oil (NSO) used as ligands were carried out on a 64-bit operating system with Windows 11 Pro edition. The chemical structures of the selected components of NSO used as ligands, whose structures have been previously described in the literature (21), were created with the ChemDraw 2D program using their SMILES, and their energy minimization was carried out with the ChemDraw 3D program. Pyruvate kinase (PK) crystal structure was taken from the protein data bank (22, 23). The grid boxes were positioned with dimensions of 40x40x40 $Å^3$ and a spacing of 0.375Å, relative to the region previously determined as the active site of PK (19). The PDB file of the PK was optimized using the Maestro 2023-4 program (24). At least 50 runs were performed for each selected compound while using standard settings for PK (PDB ID: 1A3X). In all our molecular docking studies, the Lamarckian Genetic Algorithm was preferred and results such as docking scores were obtained and presented using both the AutoDock 4.2 program (25) and AutoDock Vina program (26). For docking validation, 2-Phosphoglycolic acid (PDB ID: PGA) known as a co-crystallized ligand was re-docked onto the target site of PK, and the RMSD value has been determined to be 1.31 (Table 3).

ADME Predictions: The SwissAdme online tool (27) was used to calculate the pharmacokinetic and physicochemical properties (28-30) of some selected NSO components as ligands (a-f) (Table 4-5).

Results

***In vivo* Studies:** PK activities in the kidneys of the control group and experimental group are shown in Figure 1. When the DOX group was compared with the control group, a significant decrease in PK activity was found and a statistically significant difference in PK activity was found. Compared to the DOX-treated group, it was observed that there were significant increases in PK activity in the NSO+DOX group ($p<0.05$) and the values reached the control group values. No statistical difference was found between the control group and the NSO applied group (Figure 1).

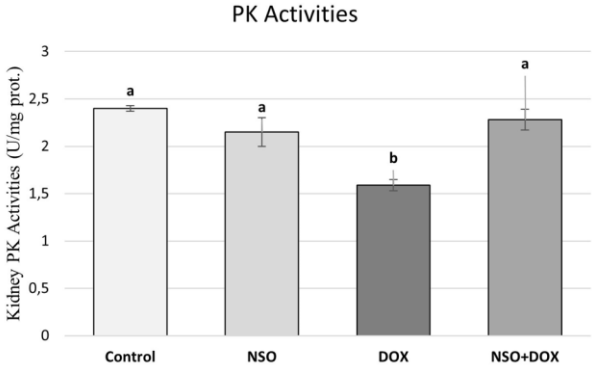


Figure 1. Effects of *Nigella sativa* oil (NSO) on kidney PK activity in DOX-treated rats

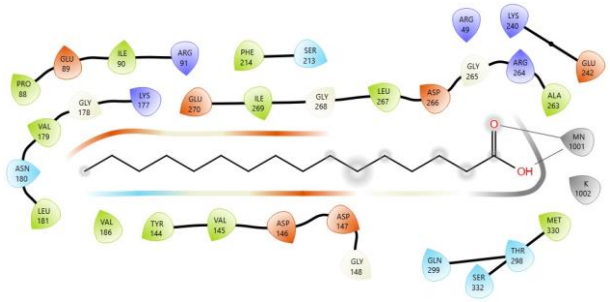


Figure 2. 2D interaction diagram with PK (1A3X) for Palmitic acid (c)

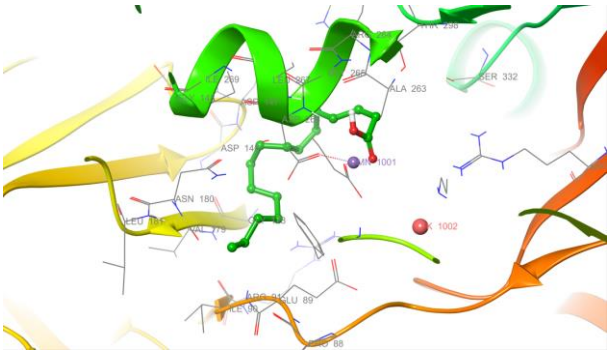


Figure 3. 3D interaction diagram with PK (1A3X) for Palmitic acid (c)

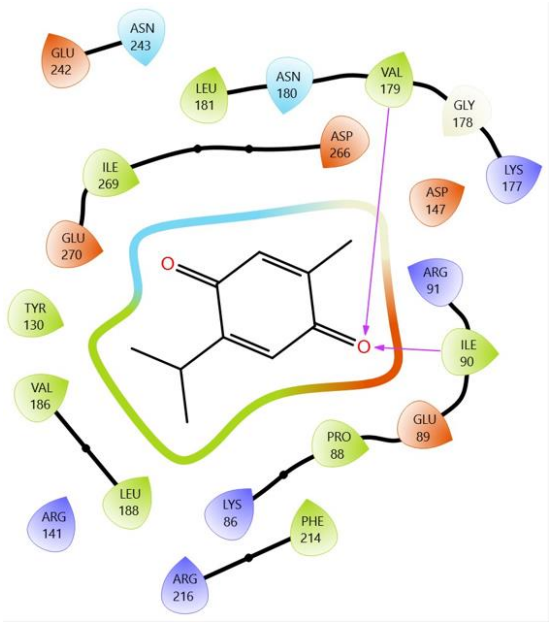


Figure 4. 2D interaction diagram with PK (1A3X) for Thymoquinone (e)

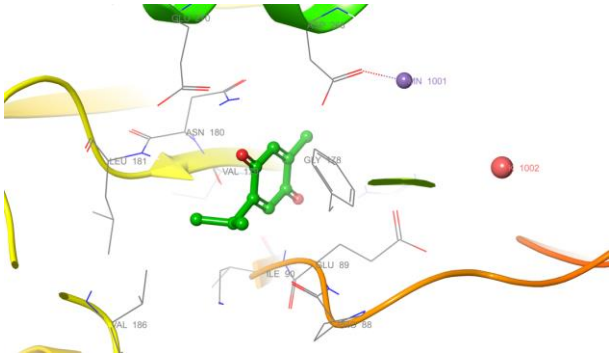


Figure 5. 3D interaction diagram with PK (1A3X) for Thymoquinone (e)

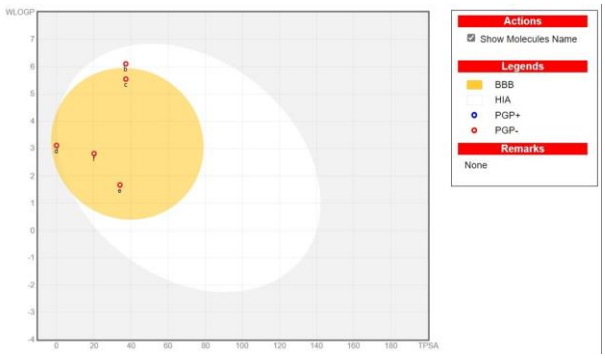


Figure 6. The BOILED-Egg model of some selected components of NSO

Molecular Docking Studies: When the docking poses of Palmitic acid (c) were examined, it was seen that it had interactions such as metal coordination. There was metal coordination between the carboxylic acid group of Palmitic acid and MN1001 (Figure 2-3). When the molecular docking poses of Thymoquinone (e) were examined, it was seen that it had interactions such as hydrogen bonds. There were H-bond interactions between the carbonyl group of Thymoquinone and ILE90 and VAL179 (Figure 4-5).

When the docking scores of Linoleic acid (a), oleic acid (b), and palmitic acid (c), which are the compounds detected in the fixed fatty acid, were examined, it was seen that inhibited PK at the nanomolar level. It was observed that *p*-cymene (d), Thymoquinone (e), and Carvacrol (f), which are the compounds detected in the essential fatty acid, inhibited PK at the micromolar level (Table 3).

Table 3. Molecular docking scores, binding types, and estimated inhibition constants of some components of *Nigella sativa* oil on PK (PDB ID: 1A3X)

Compounds	Based on Visual Results Interacting Residues		Autodock Results		Vina Results
	Hydrogen Bond	Metal Coordination	Estimated Inhibition Constant, Ki	The Best Docking Score	The Best Docking Score
Linoleic acid	ASP266	MN1001	4.57 nM	-11.38	-5.6
Oleic acid	-	MN1001	17.72 nM	-10.57	-5.2
Palmitic acid	-	MN1001	4.91 nM	-11.33	-4.5
p-Cymene	-	-	255.92 µM	-4.90	-6.0
Thymoquinone	ILE90 VAL179	-	52.05 µM	-5.84	-6.2
Carvacrol	ILE90	-	101.47 µM	-5.43	-6.0

µM: micromolar, nM: nanomolar, Docking Score: Estimated Binding Free Energy (kcal/mol)

ADME Predictions: The SwissAdme software is used to facilitate the process of becoming a drug while providing some preliminary research results on the evaluation of the drug potential of molecules. The physicochemical and pharmacokinetic properties of all selected components of NSO were evaluated appropriately to achieve drug-likeness. Log S is defined as a common unit of solubility of a drug. Except for Linoleic acid (a), for all selected compounds Log S values are between -2.18 and -5.41, and their solubility is estimated to be between soluble and moderately. The F values, which indicate the oral bioavailability of the selected components of NSO, are between 0.55-0.85. Drug-likeness, water solubility, and pharmacokinetics properties could not be determined for Linoleic acid (a) which is water-insoluble. Gastrointestinal absorption was found to be high for all compounds (except a) and low for *p*-cymene (d), with little or no absorption for compounds a and d due to the abundance of apolar groups they carry (Table 4-5, Figure 6).

Statistical Analysis: The evaluation of statistical significance between the four different groups mentioned was done using the SPSS 22 package program. The Shapiro-Wilk normality test was used to evaluate whether the raw values of all parameters showed a normal distribution, and each parameter value was found to have a normal distribution. In addition, whether the variances showed a homogeneous distribution was determined using the Levene test. Based on the outcomes of this test, group differences were assessed utilizing *post hoc* Tukey testing and one-way analysis of variance (ANOVA), which was utilized to compare the groups. All values were derived using the mean and the standard error of the mean. The mean and standard error were used to illustrate the study's conclusions. *p*-values that fell below 0.05 were considered to be statistically significant.

Table 4. Drug-likeness, water solubility, and pharmacokinetic properties of selected components of NSO

Comp.	Drug-likeness					Water Solubility		Pharmacokinetics	
	Lipinski	Ghose	Veber	Egan	Muegge	LogS	Class	GI abs.	F
a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
b	+	-	-	-	-	-5.41	Moderately	High	0.85
c	+	+	-	+	-	-5.02	Moderately	High	0.85
d	+	-	+	+	-	-3.63	Soluble	Low	0.55
e	+	+	+	+	-	-2.18	Soluble	High	0.55
f	+	-	+	+	-	-3.31	Soluble	High	0.55

LogS: ESOL, Class: -6 <Moderately <-4 GI abs: Gastrointestinal absorption, F: Bioavailability score, n/a: no answer.

Table 5. The physicochemical and lipophilicity properties of selected components of NSO

Comp.	Physicochemical Properties							Lipophilicity
	MW	Fsp3	RB	HBA	HBD	MR	TPSA	cLogP
a	280.45	0.72	14	2	1	89.46	37.30	5.88
b	282.46	0.83	15	2	1	89.94	37.30	5.65
c	256.42	0.94	14	2	1	80.80	37.30	5.20
d	134.22	0.40	1	0	0	45.99	0	3.50
e	164.20	0.40	1	2	0	47.52	34.14	1.85
f	150.22	0.40	1	1	1	48.01	20.23	2.82

Comp: Compounds, MW: Molecular weight, Fsp3: Fraction, RB: Number of rotatable bonds, HBA: Number of hydrogen bond acceptors, HBD: Number of hydrogen bond donors, MR: Molar refractivity, TPSA: Total polar surface area

Discussion

NSO has attracted great interest as a medicinal and possible preventive agent in recent years due to its numerous stated advantages. The negative effects of DOX on kidneys were examined as biochemical it was aimed to evaluate the possible effects of NSO when faced with DOX-related kidney damage. As a result, it is seen that NSO treatment significantly approaches the results of the control group in terms of PK activity. The results obtained raise the possibility that NSO could be used as an adjuvant therapy to protect organs from DOX-induced oxidative stress. Yilmaz et al. in their study aiming to evaluate the effect of propolis on PK, a key enzyme in glycolysis, and superoxide dismutase, an antioxidant enzyme, on DOX-induced toxicity in different tissues that there was a decrease in PK activities in many tissues including kidney tissue after DOX was applied at the same dose as in the current study. In a study examining changes in pyruvate kinase enzyme activities in the liver and kidney tissues of rats induced with streptozotocin, it was determined that PK activities in the kidney tissue decreased statistically insignificantly on the 3rd day of diabetes. In the same study, it was determined that PK activity decreased in the liver tissue after diabetes (19, 31). In this study, PK activity decreased after DOX application, and this decrease may be due to a decrease in pyruvate kinase synthesis. Pyruvate kinase, one of the enzymes of the glycolytic pathway, is a key enzyme in glucose metabolism. This should also suggest that DOX used in cancer treatment may cause a decrease in the gene transcription level of the PK enzyme.

In the previous study, the interaction area of PK (PDB ID: 1A3X) and its cocrystal ligand, 2-phosphoglycolic acid (PDB ID: PGA), was revealed. Hydrogen bonds were observed between ARG49, GLY265, and THR298 between PGA and the macromolecule. It was also emphasized that it was observed to form a metal coordination bond with MN1001 (32).

When the docking results were examined, it was seen that the components detected in the fixed oil interacted with MN1001, which was seen to be important in terms of interaction, but the essential oil components did not interact. When the estimated inhibition values on PK were examined here, it was estimated that fixed oil components would inhibit at the nanomolar level and essential oil components would inhibit at the micromolar level. In their recent study on pyruvate kinase, Yilmaz et al. reported that caffeic acid phenethyl ester (CAPE) makes ASP266 hydrogen bonds similar to Linoleic acid (a) (19).

The fact that the molecular volumes of the fixed oil components are relatively large and the essential oil components are relatively small may be effective in these results. Especially when the dock scores of Linoleic acid (a) were examined, it was perceived that it would have the highest affinity for the PK (Table 3). Also, it showed that Palmitic acid (c) and Thymoquinone (e) were one of the selected compounds with the best pose on the PK, for fixed and essential oils respectively (Figure 2-5).

Looking at the SwissAdme Boiled Egg model, no results were obtained for Linoleic acid (a). Only Oleic acid (b) was observed in the white zone, which stands for human gastrointestinal absorption (HIA). Palmitic acid (c), *p*-Cymene (d), Thymoquinone (e), and Carvacrol (f) were seen in the yellow region, which means passage through the blood-brain barrier. It was observed that all selected components of NSO were in full compliance with the Lipinski rules (except a). In particular, Thymoquinone (e) was found to be compatible with the rules of Lipinski, Ghose, Veber, and Egan, excluding Muegge. None of the compounds complied with the Muegge rule under the drug-likeness heading in the ADME predictions tables. Considering ADME predictions compounds b, c, d, e, and f had good pharmacokinetic profiles, but compound a was not found any drug-likeness properties (Table 4-5, Figure 6).

When the DOX group was compared with the control group, a decrease in PK activity was found, and a significant difference was found in PK activity. Moreover, it was determined that PK activity in the group administered DOX together with NSO increased statistically significantly compared to the DOX group and the values approached the control group values. When we look at the molecular docking studies conducted to elucidate this in vivo effect of NSO, it is thought that the dock score of especially the fixed oils contained in NSO is higher, but this dock score difference between the fixed and volatile oil components may depend on the structural volumes of the components. When molecular docking poses and interacting residues were evaluated together, it was concluded that the essential oil components may be more active. The results of the essential oil components with higher drug similarity rates in ADME predictions support these assumptions.

References

1. Kaya E, Yılmaz S. Çörek Otu Yağının Ratlarda Doksorubisin Kaynaklı Kardiyotoksisite Üzerindeki Etkilerinin Belirlenmesi. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi 2019; 33: 31-36.
2. Sharma NK, Ahirwar D, Jhade D, et al. Medicinal and Phamacological Potential of Nigella sativa: A Review. Ethnobotanical Leaflets 2009; 2009: 11.
3. Yaman I, Balikci E. Protective effects of nigella sativa against gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol 2010; 62: 183-190.
4. Kanter M, Demir H, Karakaya C, et al. Gastroprotective activity of Nigella sativa L oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol 2005; 11: 6662-6666.

5. Uz E, Uz B, Yusuf S, et al. Cardioprotective effects of Nigella sativa oil on cyclosporine A-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol 2008; 103: 574-580.
6. Farooqui Z, Afsar M, Rizwan S, et al. Oral administration of Nigella sativa oil ameliorates the effect of cisplatin on membrane enzymes, carbohydrate metabolism, and oxidative damage in rat liver. Toxicol Rep 2016; 3: 328-335.
7. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000; 14: 323-328.
8. Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol 2005; 5: 1749-1770.
9. Israelsen WJ, Vander Heiden MG. Pyruvate kinase: Function, regulation, and role in cancer. Semin Cell Dev Biol 2015; 43: 43-51.
10. Chhipa AS, Patel S. Targeting pyruvate kinase muscle isoform 2 (PKM2) in cancer: What do we know so far? Life Sci 2021; 280: 119694.
11. Chen DQ, Han J, Liu H, et al. Targeting pyruvate kinase M2 for the treatment of kidney disease. Front Pharmacol 2024; 15: 1376252.
12. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
13. Johnson-Arbor K, Dubey R. Doxorubicin. Treasure Island (FL): StatPearls Publishing, 2023.
14. Wallace KB. Doxorubicin-induced cardiac mitochondrionopathy. Pharmacol Toxicol 2003; 93: 105-115.
15. Yamauchi C, Fujita S, Obara T, et al. Effects of room temperature on reproduction, body and organ weights, food and water intake, and hematology in rats. Lab Anim Sci 1981; 31: 251-258.
16. Iqbal M, Dubey K, Anwer T, et al. Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. Pharmacol Rep 2008; 60: 382-390.
17. Beutler E, Blume KG, Kaplan JC, et al. International Committee for Standardization in Haematology: recommended methods for red-cell enzyme analysis. Br J Haematol 1977; 35: 331-340.
18. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
19. Yilmaz S, Kaya E, Yonar H, et al. Effect of propolis on pyruvate kinase and superoxide dismutase activities in doxorubicin-induced tissue damage: Molecular docking analysis. Rev Cient Fac Cienc Vet Univ Zulia 2024; 34: 11.
20. Sultan MT, Butt MS, Anjum FM, et al. Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. Pak J Bot 2009; 41: 1321-1330.
21. PubChem. "U.S. National Library of Medicine". https://pubchem.ncbi.nlm.nih.gov/ 15.10.2024.
22. Jurica MS, Mesecar A, Heath PJ, et al. The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate. Struct 1998; 6: 195-210.
23. RCSB. "The Protein Data Bank". https://www.rcsb.org/structure/1a3x/ 15.10.2024.
24. Schrödinger. "Maestro, Schrödinger". LLC, New York, NY, 2024.
25. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry 2009; 30: 2785-2791.
26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry 2010; 31: 455-461.
27. SIB. "Swiss Institute of Bioinformatics". http://www.swissadme.ch/ 15.10.2024.
28. Daina A, Michielin O, Zoete V. iLOGP: A Simple, Robust, and Efficient Description of n-Octanol/Water Partition Coefficient for Drug Design Using the GB/SA Approach. Journal of Chemical Information and Modeling 2014; 54: 3284-3301.
29. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports 2017; 7: 42717.
30. Daina A, Zoete V. A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. Chem Med Chem 2016; 11: 1117-1121.
31. Yılmaz S, Üstündağ B. The levels of pyruvate kinase activity in renal and hepatic tissues of rats with diabetes induced by streptozotocin. Turk J Vet Anim Sci 2002; 26: 549-553.
32. EMBL. "European Molecular Biology Laboratory". https://www.ebi.ac.uk/pdbe/entry/pdb/1a3x/bound/PGA#1005A/ 15.10.2024.