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Effect of propolis on pyruvate kinase and superoxide dismutase activities in doxorubicin–induced tissue damage: Molecular docking analysis

Efecto del propóleo sobre la actividad de la piruvato quinasa y la superóxido dismutasa en el daño tisular inducido por doxorrubicina: análisis de acoplamiento molecular

***Seval Yilmaz*[1]*, *Emre Kaya*[1], *Harun Yonar*[2], *Harun Uslu*[3]**

[1]*Firat University, Faculty of Veterinary Medicine, Department of Biochemistry. Elazig, Türkiye.*
[2]*Selcuk University, Faculty of Veterinary Medicine, Deparment of Biostatistics. Konya, Türkiye.*
[3]*Firat University, Faculty of Pharmacy, Department of Pharmaceutical Professional Sciences, Department of Pharmaceutical Chemistry. Elazig, Türkiye.*
**Corresponding author: sevalyilmaz@firat.edu.tr*

ABSTRACT

This study aimed to investigate the effect of propolis on pyruvate kinase (PK) which is a key enzyme in glycolysis and superoxide dismutase (SOD), an antioxidant enzyme on toxicity induced by DOX in different tissues. Using molecular docking, It was looked into how propolis affected the enzymes responsible for glycolysis and the antioxidant system. There was no application in the first group (control). The second group received 100 mg·kg^{-1} day of propolis by gavage needle for 7 days, a single dose of 20 mg·kg^{-1} intraperitoneal DOX to the third group, and propolis+DOX to the fourth group. Two days prior to DOX administration, propolis application began, and it lasted for seven days. PK and SOD activities were determined in liver, heart, kidney, and testis tissues, and molecular docking was applied to ratify the activity of some propolis components (caffeic acid phenethyl ester (CAPE) and Quercetin) on PK and SOD enzymes. When the DOX group was compared with the control group, a decrease in PK and SOD activities were found, and significant difference was found in PK and SOD activities. Administration of DOX decreased PK and SOD activities of liver, heart, kidney, and testis tissues. In conclusion, our study reveals that DOX disrupts glycolysis in rat tissues. CAPE and Quercetin compounds were shown to interact similarly with the cocrystal ligands of PK and SOD. In addition, when the interaction types of these compounds especially on PK and the docking scores obtained were examined, it can be said that they show higher affinity than DOX.

Key words: Doxorubicin; toxicity; pyruvate kinase; superoxide dismutase; molecular docking

RESUMEN

El estudio tuvo como objetivo, evaluar el efecto del propóleo sobre la piruvato quinasa (PK), que es una enzima clave en la glucólisis y la superóxido dismutasa (SOD), una enzima antioxidante sobre la toxicidad inducida por DOX en diferentes tejidos. Mediante el acoplamiento molecular, analizamos cómo afectaba el propóleo a las enzimas responsables de la glucólisis y el sistema antioxidante. No hubo solicitud en el primer grupo (control). El segundo grupo recibió 100 mg·kg^{-1} día de propóleo por sonda gástrica durante 7 días, el tercer grupo recibió una dosis única de 20 mg·kg^{-1} de DOX intraperitoneal y el cuarto grupo propóleo+DOX. Dos días antes de la administración de DOX, se inició la aplicación de propóleo, que duró siete días. Se determinaron las actividades de PK y SOD en tejidos de hígado, corazón, riñón y testículos, y se aplicó acoplamiento molecular para ratificar la actividad de algunos componentes del propóleo (éster fenetílico del ácido cafeico (CAPE) y quercetina) sobre las enzimas PK y SOD. Cuando se comparó el grupo DOX con el grupo de control, se encontró una disminución en las actividades de PK y SOD, y se encontró una diferencia significativa en las actividades de PK y SOD. La administración de DOX disminuyó las actividades de PK y SOD de los tejidos del hígado, el corazón, los riñones y los testículos. En conclusión, el presente estudio revela que DOX interrumpe la glucólisis en tejidos de rata. Se demostró que los compuestos CAPE y quercetina interactúan de manera similar con los ligandos cocristalinos de PK y SOD. Además, cuando se examinaron los tipos de interacción de estos compuestos, especialmente en PK, y las puntuaciones de acoplamiento obtenidas, se puede decir que muestran mayor afinidad que DOX.

Palabras clave: Doxorrubicina; toxicidad; piruvato quinasa; superóxido dismutasa; acoplamiento molecular

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INTRODUCTION

Doxorubicin (DOX), also known as Adriamycin®, is an active chemotherapy medication used to treat a variety of cancers, including uterine, ovarian, lung, and breast cancers. However, its clinical efficacy is constrained by significant toxicities, such as cardiac, hepatic, renal, pulmonary, hematological, and testicular harm [1].

The rate-limiting enzyme of glycolysis known as pyruvate kinase (PK), is essential for the metabolism of cancer cells [2]. Most cancers utilize glucose substantially more than normal tissue does. Aerobic glycolysis, also known as increased glucose consumption and elevated lactate generation in the presence of oxygen, is more prevalent in cancer cells (the Warburg effect)[3, 4], of which PK is considered a key regulator. An enzyme called PK catalyzes the last step of glycolysis by phosphorylating adenosine diphosphate (ADP) to adenosine triphosphate (ATP) and converting phosphoenolpyruvate to pyruvate. Previous research has shown that PK is essential for cell cycle progression, tumor growth, maintenance of the malignant phenotype, and cell migration. The major multi-ability enzyme PK still has unidentified activities in malignancies [2, 5].

Some of the postulated causes of DOX toxicities include oxidative stress, inflammation, endoplasmic reticulum-mediated apoptosis, and DNA / RNA damage. Superoxide ($O_{2}^{\cdot-}$) and hydroxyl radicals (OH·) are produced as a result of the cytochrome P-450 enzyme's metabolism of the DOX, which damages cellular membranes [6]. After passing through the cell membrane and being reduced by cellular flavoenzymes, the drug DOX causes an increase in the production of intracellular free radicals [7]. Reactive oxygen species (ROS) are produced by disrupting complex I of the mitochondrial electron transport chain (ETC), which is DOX-mediated redox cycling, according to a number of methods. When reactive species are present, macromolecules (such as lipids, proteins, DNA, etc.) undergo oxidative changes that have harmful effects[8, 9]. The increase in ROS generation, particularly $O_{2}^{\cdot-}$ and $OH^{\cdot}$, leads to tissue damage from DOX as well as certain non-radical substances including singlet oxygen ($^{1}O_{2}$), hydrogen peroxide (H_2O_2), and others [10, 11]. The myocardium typically causes DOX poisoning, which eventually destroys other organs [12]. The heart's work consumes a lot of ATP, which is produced by a variety of metabolic processes using glucose, free fatty acids, pyruvate, and ketone bodies. The heart may also adjust to variations in the fuel supply. DOX slows lipogenesis, which then prevents lipolysis [12, 13]. Overall, it is clear that the ATP produced by the metabolism of fatty acids may have been altered, forcing the cardiomyocytes to move to alternative substrates to produce ATP, such as glycolysis [14].

Normally, intracellular enzymes including glutathione reductase, superoxide dismutase (SOD), and catalase detoxify ROS [15]. SOD facilitates the dismutation of $O_{2}^{\cdot-}$ into either H_2O_2 or regular molecular oxygen (O_2). As a result, SOD provides excellent antioxidant protection in almost all live cells exposed to oxygen [16].

By incorporating a protective agent into DOX treatment methods, numerous strategies have been developed to reduce these adverse effects. Based on these beliefs, numerous antioxidants, anti-inflammatory, and anti-apoptotic medications have been recommended to combat produced damage sales [17, 18].

Honeybees (*Apis mellifera L.*) gather propolis, often known as bee glue, from the leaf buds and bark fissures of many different types of trees [19]. The composition and effectiveness of propolis varies widely depending on its source, location, environment, and age. It contains more than 150 polyphenols, including their esters, and flavonoids like phenolic acid. The flavonoids in propolis are abundant and exhibit potent free radical scavenging properties. Additionally, it includes several vital minerals, including Ca, Zn, Mg, Cu, Mn, Fe, and Ni, as well as vitamins E and C, vitamin B complexes, certain elements, and other vitamins. As a result, it demonstrates a variety of biological functions, including actions that are antibacterial, antioxidant, and capable of scavenging free radicals [20, 21]. Propolis has recently been found to exhibit a variety of biological activities, including those that are antibacterial, antifungal, antiviral, immunoregulatory, antioxidative, anticancer, hepatotropic, and antiinflammatory, as well as potential use for coronavirus 2019 (COVID-19)[22, 23]. The results indicate that CAPE-like compounds could be employed as possible chemotherapeutic agents against oral cancer. Additionally, oral submucosal fibroblast, neck gingival carcinoma, and tongue squamous cell carcinoma cells were revealed to be highly cytotoxic to CAPE [24]. The widely dispersed flavonoids are quercetin and rutin, which are significant representatives of the biologically active components in propolis and have strong antioxidant and anticancer effects [25].

One can describe the behavior of ligands and target proteins at the binding site and understand biochemical processes by using molecular docking to mimic the interaction between a ligand and a protein [26]. For molecular docking investigations, CAPE and Quercetin compounds were chosen because they have different chemical structures, have a greater proportion of propolis components, and exhibit similar activity to these molecules [27].

It was aimed to investigate the effect of propolis on pyruvate kinase and superoxide dismutase activity in doxorubicin-induced tissue damage. The molecular docking study was planned to analyze to understand the interaction between DOX and enzymes. Due to its broad range of biological activity, propolis has recently been increasingly used in foods and beverages to promote health and prevent diseases. This evaluation included the biochemical, molecular docking chain that uses rats to investigate propolis mechanism *in vivo* against tissue toxicity induced by DOX as an antioxidant, inflammatory and antiapoptotic agent.

MATERIALS AND METHODS

Experimental design

The local animal experiment ethics committee of Firat University gave its consent to the conduct of this investigation (Protocol No: 2012/03-43). Forty-eight, three-month-old male Wistar-Albino rats (*Rattus norvegicus*) weighing 250-300 g were utilized in the study; they were procured from the Firat University Laboratory Animals Breeding Unit. The rats were kept in air-conditioned rooms with a fixed temperature of 25 ± 2°C and 60-65% humidity, with a 12/12h light/dark cycle, under standard conditions, and were fed on standard rat food (pellet) and tap water *ad libitum* throughout the experimental practices [28]. Experimental practices on rats were performed in the Firat University Experimental Research Center.

In the study, rats were put into 4 groups, each including 7 rats: 1st group: control group, 2nd group: the group that received 100 mg·kg^{-1} day (d) propolis by gavage for 7 d, 3rd group: the group that received single dose 20 mg·kg^{-1} body weight DOX (Fresenius Kabi Oncology Ltd. 19 Industrial Area, Baddi, Distt. Solan-India) intraperitoneally, and 4th group: the group that received propolis (100 mg·kg^{-1} d by gavage, 7 d) + DOX (20 mg·kg^{-1} body weight, intraperitoneal single dose). Propolis

application was started 2 d before DOX administration and continued for 7 d. The amount of DOX used in the study was determined based on the previous studies [29, 30]. Rats were sacrificed by decapitation method 5 d after DOX administration in DOX treated group, rats in the control and propolis groups, start of the experiment were sacrificed after 7 d, in the DOX+Propolis group, propolis pre-treatment was performed for 2 d, then DOX administrated, were sacrificed by decapitation method 5 d after DOX administration. The activities of PK and SOD enzymes were determined spectrophotometrically (Thermo Scientific, Genesys 10S UV-VIS Spectrophotometer, USA) in the liver, heart, kidney and testis tissues.

Biochemical analysis

At the conclusion of the experiment, the rats were slaughtered, and tissue samples of the liver, heart, kidney, and testis were collected. Until biochemical analysis, tissue samples were kept at -80 °C in a freezer. Physiological saline solution was used to wash the tissue samples, and they were subsequently diluted with distilled water at a weight-to-volume ratio of 1:10 and homogenized in a Potter-elvehjem homogenizer (CAT R50D, Germany). Centrifugation (NUVE NF800R, Turkey) was done on homogenates at +4 °C for 15 minutes at 3,500 rpm for SOD activity analysis, and for 55 min at 13,500 rpm for PK activity analysis.

The PK activity were measured spectrophotometrically using the method modified from Beutler *et al*. [31], which is based on measuring the decreasing absorbance rate of NADH at 340 nm. SOD activity was determined according to the method modified by Sun *et al*. [32]. SOD activity was measured using the method based on the measurement of color development upon the reduction of nitroblue tetrazolium by the $O_2\cdot^-$ produced by the xanthine-xanthine oxidase system. The Lowry *et al*. [33] method was used to determine the protein concentration in tissue homogenate.

Molecular docking

The chemical structures of the selected propolis components were obtained from the internet in the form of SMILES (https://pubchem.ncbi.nlm.nih.gov/). Energy minimization of ligands were performed using ChemOffice software. *In silico* study was performed with Autodock4 program to validate the *in vivo* tests of propolis on PK and SOD. Grid box points as different Å^3 size and 0.375 Å regular spacing were made by centering separately for active sites of PK and SOD enzymes previously determined or predicted. Pdb file of enzymes were get (https://www.rcsb.org/) and were optimized using the Maestro program (Maestro, Schrödinger, LLC, New York, NY, 2020). The 50-run Lamarckian Genetic Algorithm was used, while standard settings were used for all ligands. The molecular docking tool AutoDock 4.2 was used to calculate docking scores [34]. Cocrystal was redocked on the SOD enzyme in order to validate the docking algorithm, and the RMSD value was found to be 1.33.

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics (Version 22) and R 4.2.2 (R Core Team, 2021)(https://www.r-project.org/). Numerical variables were reported as means, standard deviations, medians, and interquartile ranges (IQR). Normality was assessed using the Kolmogorov-Smirnov/Shapiro-Wilk tests. Differences in measured parameters were analyzed using the Kruskal-Wallis test. Pairwise differences were evaluated using the Mann-Whitney U test with Bonferroni correction. The Spearman test was performed to identify relationships between the measured parameters. A significance level of $P<0.05$ was used for all analyses.

RESULTS AND DISCUSSIONS

The TABLE I presents the PK and SOD activities in the liver, heart, kidney and testis of the control and experimental groups. When the DOX group was compared with the control group, a significative decrease in PK and SOD activities were found, and a statistically significant difference was found in PK and SOD activities. Compared to the DOX-treated group, it was observed that there were significant increases in both PK activities and SOD activities in the DOX-administered propolis group ($P<0.05$) and the values reached the control group values (TABLE I).

Correlation between PK and SOD in the liver tissue

A positive correlation of nearly 100% was found between PK and SOD activities in liver tissue (FIG. 1). As PK activity decreases, SOD activity also decreases. A positive correlation of nearly 100% was found between PK and SOD activities in the DOX+propolis group. As PK activity increases, SOD activity also increases. In terms of SOD activity, an inverse relationship of 97% was found between the DOX group and the DOX+Propolis group (FIG. 2).

TABLE I
Effects of propolis on the PK and SOD activities in liver, heart, kidney and testis tissues of DOX treated rats

	Activities	Control		Propolis		Doxorubicin		Doxo.+Prop.		*P*
		Mean±SD	Med(IQR)	Mean±SD	Med(IQR)	Mean±SD	Med(IQR)	Mean±SD	Med(IQR)	
PK	Liver (U·mg^{-1} Protein)	10.80±2.95	9.49 (5.03)^{a}	11.31±2.09	11.63 (4.80)^{a}	6.80±1.21	7.31 (2.24)^{b}	9.39±1.47	8.98 (2.06)^{a}	<0.001
	Kidney (U·mg^{-1} Protein)	2.37±0.15	2.34 (0.09)^{a}	2.58±0.55	2.83 (1.03)^{a}	1.91±0.20	2.04 (0.30)^{b}	2.22±0.43	2.33 (0.87)^{ab}	<0.001
	Heart (U·mg^{-1} Protein)	5.03±1.67	5.54 (3.32)^{ab}	5.79±1.04	5.86 (2.08)^{a}	3.81±0.86	3.78 (1.36)^{b}	5.05±1.18	4.70 (1.47)^{ab}	<0.001
	Testis (U·mg^{-1} Protein)	6.39±0.73	6.54 (1.62)^{a}	6.45±0.22	6.43 (0.42)^{a}	4.13±2.27	5.03 (4.61)^{b}	6.24±0.90	6.48 (1.24)^{a}	<0.001
SOD	Liver (U·mg^{-1} Protein)	0.19±0.03	0.18 (0.06)^{ac}	0.204±0.02	0.20 (0.02)^{a}	0.14±0.04	0.16 (0.07)^{bc}	0.17±0.02	0.17 (0.04)^{c}	<0.001
	Kidney (U·mg^{-1} Protein)	0.22±0.04	0.22 (0.09)^{a}	0.216±0.02	0.21 (0.03)^{a}	0.19±0.01	0.18 (0.03)^{b}	0.22±0.02	0.22 (0.03)^{a}	<0.001
	Heart (U·mg^{-1} Protein)	2.52±0.24	2.47 (0.50)^{a}	2.62±0.27	2.76 (0.32)^{a}	2.06±0.48	2.28 (0.97)^{b}	2.52±0.13	2.53 (0.25)^{a}	<0.001
	Testis (U·mg^{-1} Protein)	0.14±0.06	0.13 (0.14)^{a}	0.13±0.05	0.15 (0.11)^{ab}	0.09±0.04	0.08 (0.06)^{b}	0.13±0.05	0.13 (0.11)^{a}	<0.001

The different letters in the rows indicate a statistically significant difference between the groups and *P* values are for Kruskall-Wallis test. Significance values of multiple comparisons have been adjusted by the Bonferroni correction

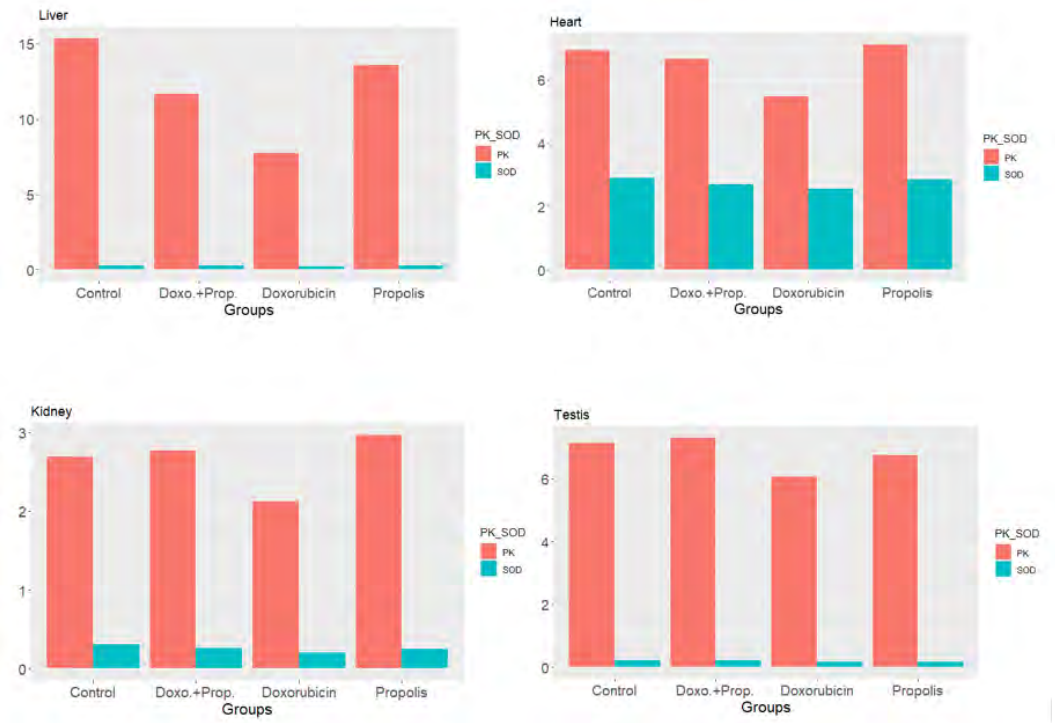

FIGURE 1. Bar graphs of PK and SOD values of each tissue in terms of groups of interest

Correlation between PK and SOD in the heart tissue

A statistically insignificant positive correlation was found between PK and SOD activities in heart tissue. A very low correlation was found between PK and SOD activities in the DOX+Propolis group (FIGS. 1, 2).

Correlation between PK and SOD in the kidney tissue

An inverse correlation of 0.50% was found between PK and SOD activities in kidney tissue. While PK activity increased, SOD activity decreased. The relationship between PK and SOD activities was not significant in the DOX+Propolis group (FIGS. 1, 2).

Correlation between PK and SOD in the testis tissue

A positive correlation of 48% was found between PK and SOD activities in the DOX group in testicular tissue. As PK activity decreases, SOD activity also decreases. There was a 68% negative correlation between PK activity in the DOX group and SOD activity in the DOX+propolis group. The relationship between PK and SOD activities in the DOX+propolis group was not statistically significant (FIGS. 1, 2).

The liver is the tissue most affected by DOX application in terms of PK and SOD activities, followed by testis (FIGS. 1, 2).

Molecular docking analyses were carried out to ascertain the modes of interaction at the active sites of these enzymes for some propolis components and docking scores were obtained (TABLE II). It showed higher affinity for PK enzyme than DOX, with higher binding scores and similar interaction modes of CAPE and quercetin. In another enzyme, SOD, DOX was found to have a higher binding score than CAPE and quercetin. The related amino acids and binding types were shown in detail in 2D and 3D figures by the Maestro program. The interactions of PK and SOD enzymes, whose active site was previously defined or predicted, with CAPE, Quercetin and DOX are presented in detail (FIGS. 3, 4, 5, 6, 7, 8, 9).

The structures of PK as determined by X-ray crystallography and SOD main binding sites have been determined (https://www.rcsb.org/) [35]. It has been previously represented that 2-phosphoglycolic acid (PDB ID: PGA), which is a cocrystal in PK (PDB ID: 1A3X), bonds hydrogen ARG49, GLY265 and THR298. It has been previously represented covalent metal complex interaction with MN1001 for PGA on PK (https://www.ebi.ac.uk/pdbe/). Quercetin has been found to form H-bonds with ARG49 and metal coordination with MN1001 in this instance, similar to PGA. In addition, Quercetin has been detected to make Pi cation interaction with K1002. In order to determine the likely binding model of propolis components (CAPE, Quercetin) and DOX into the active site of SOD, molecular docking studies were carried out. It has been previously represented that SOD (PDB ID: 3LSU) binding site includes HIS172, GLN179, MLY181 and ASP184 (https://goo.su/7qZOt1). CAPE interacted with GLN179, MLY181 and ASP184 and, Quercetin interacted with HIS172 and MLY181, these results showed that they were compatible with the previously shared binding site and that they interacted similarly (FIG: 3, 4, 5, 6, 7, 8 and 9)(TABLE II).

It is evident from our molecular docking observations that DOX has a detrimental effect on PK catalytic activity. Given the decreased ATP and accumulation of glycolytic intermediates, it is logical to hypothesize that glycolysis may be imperfect and cause a metabolic meltdown [16]. It's feasible that a deficiency in the availability of pyruvate caused by a dysfunctional PK could prevent acetyl CoA from being available as a substrate for the TCA cycle or the lipogenic pathway [13, 36].

The administration of propolis reduced liver, heart, kidney, and testis damage in a manner similar to the biochemical results, according to the results of molecular docking. Propolis pre-treatment

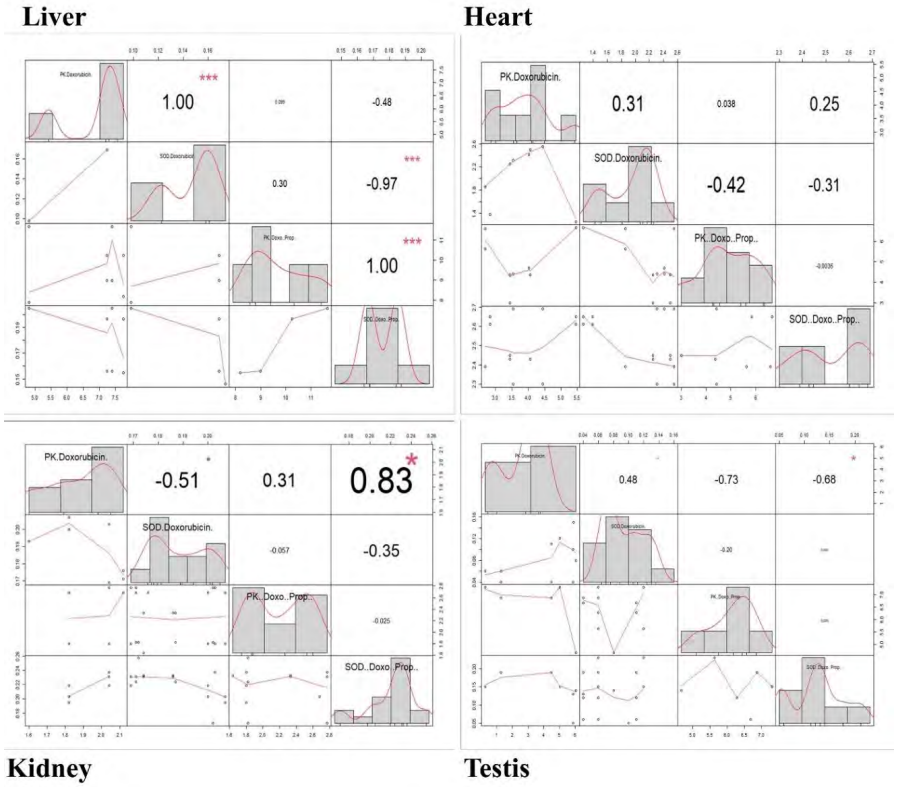

FIGURE 2. Spearman correlation values and scatter plots of PK and SOD enzyme activities results for the groups of interest of each tissue

for two days before to DOX results in substantial cause decorating in all prior markers DOX group, suggesting that propolis assisted in maintaining membrane integrity and prevented enzyme leakage. Additionally, propolis antioxidants contain polyphenols that may guard against oxidative cardiac, hepatic, and renal harm.

The negative effects of DOX on several organs were examined as biochemical and molecular dockening, and it was aimed to evaluate the possible effects of propolis, which has the potential for multiple life when faced with DOX toxic damage. Due to its numerous stated advantages and affordability, propolis has gained a lot of favor as a medicinal and possible protective agent in recent years.

TABLE II
Molecular docking binding scores of DOX, CAPE and Quercetin with PK and SOD enzymes binding residues

Enzymes	Ligands	Visualization Results of Docking				Autodock Results	
		H-Bond	Metal Coord.	Pi Cation	Pi Stacking	Estimated Inhibition Constant, Ki	Best Docking Score
PK (1A3X)	DOX	–	MN1001	–	–	277.60 µM	-4.85
	CAPE	ASP147 ASP266	MN1001	–	–	194.32 µM	-5.06
	Quercetin	ARG49 HIS5	MN1001	K1002 LYS240	–	75.85 µM	-5.62
SOD (3LSU)	DOX	D:ASP6 D:LEU7 D:PHE11	–	–	–	10.60 µM	-6.79
	CAPE	C:GLU171	–	–	D:HIS30	37.46 µM	-6.04
	Quercetin	C:GLU171 D:MLY29*	–	–	D:HIS30	89.70 µM	-5.52

µM: micromolar, Docking Score: Estimated Free Energy of Binding (kcal·mol^{-1}), MLY: Modified residue of LYS, MN: Mn^{2+}, K: K^{+}

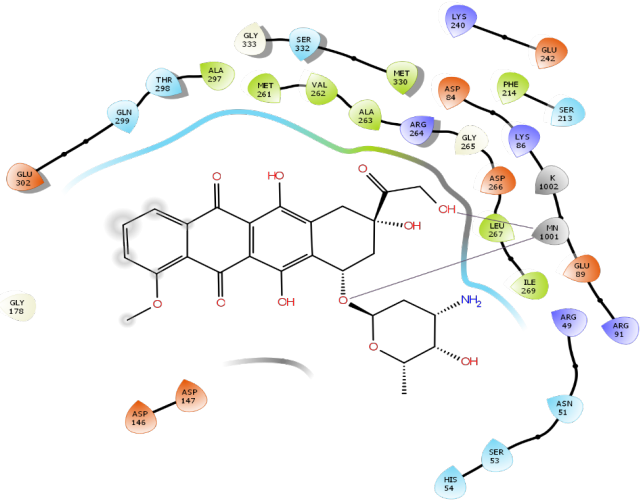

FIGURE 3. DOX is presented in the PK binding site with 2D

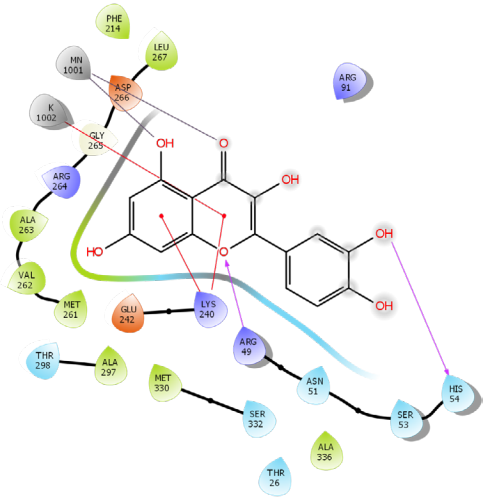

FIGURE 6. Quercetin is presented in the PK binding site with 2D

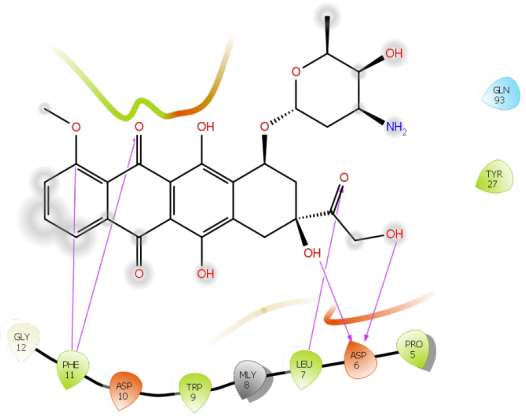

FIGURE 4. DOX is presented in the SOD binding site with 2D

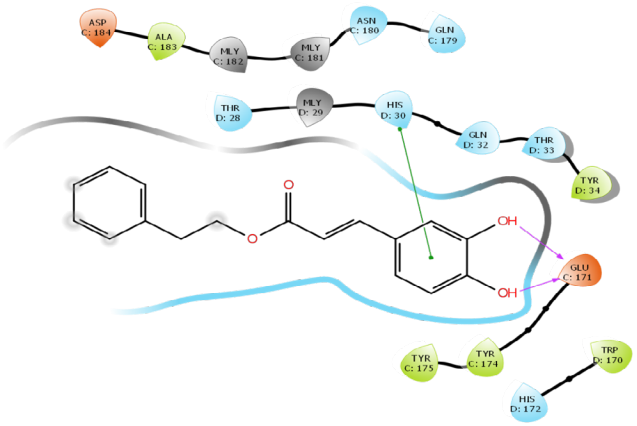

FIGURE 7. CAPE is presented in the SOD binding site with 2D

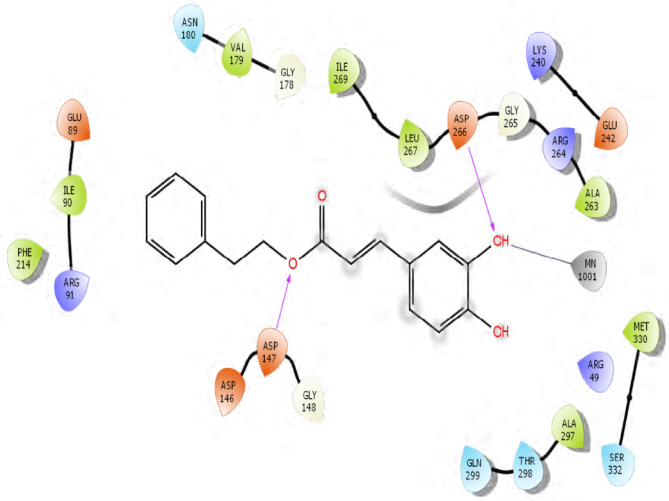

FIGURE 5. CAPE is presented in the PK binding site with 2D

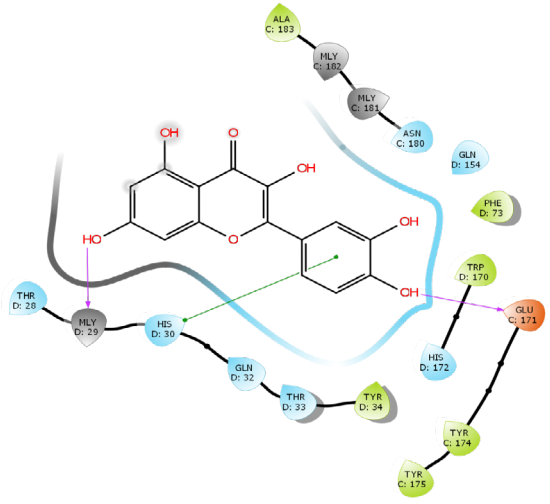

FIGURE 8. Quercetin is presented in the SOD binding site with 2D

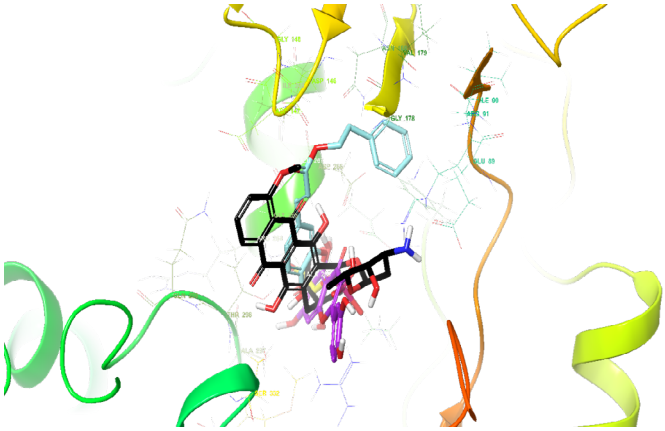

FIGURE 9. PGA (yellow), CAPE (cyan), Quercetin (purple), and DOX (black) are presented in the PK (PGA, Mn^{2+} and K^{+} complex binding site) with 3D

Since 1969, DOX, also known as Adriamycin® or Doxilthe®, has been the most widely used and successful chemotherapeutic medication for the treatment of a variety of malignancies, including solid and hematogenous cancers [37]. Despite being a powerful active anticancer medication, DOX's therapeutic use is constrained by its side effects [38].

Glycolytic enzymes enable the switch from oxidative phosphorylation to aerobic glycolysis [39]. Particularly PK, glycolytic enzymes have crucial roles in the proliferation of cancer cells. Phosphoenolpyruvate is converted into cytosolic pyruvate by PK, which also simultaneously produces one ATP molecule [40]. Pyruvates generated in the cytoplasm are headed for oxidative decarboxylation to create acetyl CoA, which is then either used to power the citric acid cycle or another metabolic route. Previous studies showed that DOX inhibits adipogenic and lipogenic pathways [12, 13]. It was therefore tried to find out how DOX affected the glycolytic process. According to gene expression studies, PK is downregulated by DOX, which results in dysregulation of glycolysis. Glycolysis disruption may result in a reduction in ATP synthesis and energy deprivation in cells [41].

As a model organism, Mohan *et al.* [16] treated *Saccharomyces cerevisiae* with PK at varying concentrations (5–50 µM) to examine the impact of DOX on the glycolytic pathway and apoptosis. As medication concentration rose, a decline in growth rate was seen. The biphasic character of DOX was revealed by an increase in cell density at the highest dose (50 µM). Mohan *et al.* [16] reveal that Adriamycin disrupts glycolysis in yeast cells, causing the cell to undergo apoptosis due to oxidative stress. In the current study, the change in PK activities, an important enzyme in the glycolytic pathway, after DOX (Adriamycin) application between groups supports this. The maximal growth rate was observed to be decreased by the PK concentration from 0.3 to 0.1 OD h^{-1} at 20 µM. At a 50 µM concentration, it was found that more than 80% of yeast cells were still alive [42].

After treatment, acquired drug resistance poses a significant issue for DOX and other chemotherapeutic drugs. miR-122 expression levels were found to be reduced in DOX-resistant Huh7/R cells compared to wild-type cells by Pan *et al.* [43], proving that miR-122 is connected to DOX chemoresistance. High levels of miR-122 expression in Huh7/R cells have been demonstrated to reverse DOX resistance by inhibiting PK, causing DOX-resistant cancer cells to undergo apoptosis. As a result, it has been shown that upregulating glucose metabolism makes people more resistant to DOX; as a result, miR-122's restriction of glycolysis may be a useful therapeutic approach to combat DOX resistance in liver cancer. Pan *et al.* [43] revealed that dysregulated glucose metabolism contributes to DOX resistance and inhibition of miR-122–induced glycolysis may be a promising therapeutic strategy to overcome doxorubicin resistance in hepatocellular carcinoma. The decrease in PK activity observed in all tissues with DOX application in the current study is related to this.

Cardiovascular damage attributable to dosage is one DOX drawback. 80% of the heart energy comes from lipids, with the remaining 20% coming from other sources like glucose. Previous studies have demonstrated that DOX inhibits cardiomyocyte fatty acid oxidation, and that as a result, only a change in the substrate occurs [44]. Since the medication prevents –oxidation of acids, glucose is employed in this situation rather than fatty acids as the substrate [14]. The amount of mitochondria in cardiomyocytes is typically 35–40% more than that in other organs. Consequently, altering the ultrastructure of the mitochondria can impair ATP synthesis [45].

Our study showed that propolis leads to increase activities of PK. This shows that propolis has the ability to control glucose metabolism, increasing PK activity. According to our findings, administering propolis along with DOX treatment improves glucose metabolism [46, 47].

Because the aerobic organism uses oxygen to produce energy, it produces a variety of free radicals and other ROS. As a response, cells have evolved an antioxidant system that can stop and reverse ROS-mediated damage. SOD is a highly conserved enzyme that is found in all living things. Its primary job is to turn the O_2^- produced during respiration into O_2 and H_2O_2, which is crucial for detoxification [48].

Several flavoenzymes, such as NADH dehydrogenase and NADPH cytochrome P-450 reductase, can enzymatically convert DOX to its semiquinone radicals [49]. Superoxide anion radical is created when DOX radical gives up its electron to O_2 in an aerodynamic environment [50]. $O_2^{\cdot-}$ then can dismutate to H_2O_2 and/or participate in the formation of highly toxic OH via the Haber–Weiss reaction cycle. Specifically, exposure to DOX has been shown to increase intracellular H_2O_2 levels. These causes this stress by receiving electrons from the lipids in cell membranes, leading to lipid pleading toon, and oxidant-induced cell injuries [17].

In the current study, as described by Ciaccio *et al.* [17], changes in the activity of SOD, an antioxidant enzyme, after DOX application prove that the SOD enzyme catalyzes the reaction of oxidizing the superoxide radical to molecular oxygen and reducing another superoxide radical to H_2O_2. The availability of O_2 and NADPH, as well as the activity of many intracellular enzymes like SOD, glutathione peroxidase, NADPH oxidases, and thioredoxin, are all necessary for DOX to conduct the reductive conversion. The $OH^{\cdot}$ that is formed secondarily has the potential to damage proteins and DNA as well as start the process of lipid peroxidation (LPO), which has harmful effects on tissues and cells. The cytotoxicity of DOX on malignant cells and its detrimental effects on various organs are also a result of nucleotide base intercalation and cell membrane lipid binding activities.

Sarvazyan *et al.* [7], in their study examining the effects of doxorubicin on cardiomyocytes with reduced SOD levels, obtained

results that coincide with the results in the current study and concluded that, as in the current study, a significant part of the cytotoxicity of DOX can be explained by the formation of superoxide anion and that the level of intracellular SOD activity is important for cell protection. Researchers concluded that it should be taken into consideration as a factor. Additionally, the enzyme topoisomerase II, which is essential for DNA replication, is inhibited by the DOX, which also intercalates into the cellular DNA [51]. Damage to cells is caused by DOX because it interacts strongly with cellular nuclei and intercalates with DNA bases to form DOX–DNA complexes [52]. By causing ROS like H_2O_2 and $O_2^{\cdot-}$ to develop, DOX destroys malignant cells. It has been suggested that the reductive reduction of DOX is the primary determinant of DOX cytotoxicity and the underlying process determining drug resistance in cancer cells [53]. Although biomarkers of DNA damage were not determined in the current study, changes in PK and SOD activity after DOX administration suggest that there are changes in both the glycolytic pathway and the oxidative mechanism. In the present work, we determined changes in PK and SOD enzyme activities after DOX application. In their study, Swamy *et al.* obtained results similar to our study after DOX application. They suggested that DOX causes this by causing oxidation of fatty acids, disturbance in myocardial adrenergic signaling/regulation, and cellular toxicity, which leads to depression of energy metabolism in cardiac tissue [54].

Numerous research conducted over the past few decades have exhibit the propolis and its compounds extensive medicinal potential. Propolis and its phytochemicals have been shown to have antioxidant, antibacterial, antiviral, antifungal, anti–inflammatory, antiproliferative, and anticancer properties, and the list is expanding [57, 58, 59, 60, 61]. But nothing is understood about how propolis might impact tumor cells' glycolysis

In the present work, tissues exposed to DOX exhibit decreased SOD enzyme activity. The obtained results recommend the function of SOD in reducing intracellular oxidative stress and protecting rat tissues. Some researchers have demonstrated in experimental models that such tissue damage may be at least somewhat related to increased oxidative stress, which is characterized by increased free radical production or decreased endogenous antioxidant activity [62]. Xanthine oxidase/xanthine systems, H_2O_2, glucose oxidase/glucose, and isolated adult rat cardiomyocytes with normal and reduced Cu/Zn SOD activity are similarly sensitive to extracellularly produced oxidants, according to research by Sarvazyan *et al.* [7]DOX was administered to myocytes that had decreased SOD activity. The formation of an $O_2^{\cdot-}$ inside the cell is thought to be the cause of DOX's cardiotoxicity. It is still unclear why DOX is toxic to the kidneys, but possible causes include an unbalanced oxidant–antioxidant system, the production of free radicals, oxidative damage to biological macromolecules, membrane LPO, and protein oxidation [63].

In the current study, decreased SOD activity in the hepatic, cardiac, renal, and testis tissues of the DOX group suggested elevated oxidative stress. Prior to DOX therapy, the liver, heart, kidneys, and testis of animals receiving propolis had higher SOD activity. Propolis treatment decreased the production of free radicals, which would have been the cause of this. Due to an increase in DOX content in the liver throughout the detoxification process, the effects of DOX toxicity were more severe in life compared to the heart. While in the heart, the same cause propolis therapy failed to lessen this concentrated liver. In line with the obtained findings, recent research on rats exposed to DOX found that their liver, heart, kidney, and testis tissues had dramatically reduced SOD activity [62, 63, 64, 65]. In addition, DOX–induced hepatotoxicity in rats was reduced by hepatic SOD, as has already been noted in a number of investigations [66, 67]. In other respects, the PK and SOD activities in the group 4, were all discovered to be very similar to those seen in the control group. This can be explained by the ability of propolis to reduce free radical activity and strengthen the antioxidant defense system. Propolis may have a protective effect because its phenolic components maintain the structural and functional integrity of the tissue, reducing oxidative tissue damage. Propolis's antioxidant of these polyphenols, which when combined with DOX, would increase the antitumor and prevent a number of damages brought on by DOX, may be responsible for the protective actions of propolis.

Rizk *et al.* [46] shown that pretreatment with propolis could successfully reduce the toxic effects of DOX on the testes without compromising the drug's anticancer effectiveness. These findings prompted speculation that propolis might function as an adjuvant therapy, possibly shielding the testes from the oxidative and apoptotic effects of DOX and ultimately aiding in the prevention of this severe detrimental effect of DOX in clinical practice.

Fuliang *et al.* [68] demonstrated how propolis extracts boosted SOD activity. This implies that propolis may alter the metabolism of blood lipids, resulting in a reduction in LPO outputs and the scavenging of free radicals. This agrees with our results, as DOX administration reduces PK and SOD, while propolis administration prior to DOX administration reduces oxidative stress and increases glucose metabolism. Propolis may have a protective effect because its phenolic components shield tissue's structural and functional integrity and stop oxidative tissue damage.

In a study by Köse *et al.* [56], renal oxidative stress and excessive free radical emission were demonstrated to cause nephrotoxicity, which is consistent with the findings of our investigation. Propolis' nephroprotective activity is evaluated by Baykara *et al.* [66] by lowering serum urea and enhancing kidney oxidation. According to a study by Promsan *et al.* [70], pretreatment with the primary flavonoid of propolis, pinocembrin (5,7–dihydroxyflavone), improved kidney function and decreased oxidative stress and apoptotic conditions. According to these results, pinocembrin protects against nephrotoxicity, which may be at least in part because of its antioxidant and antiapoptotic properties. By reducing the rise in oxidative stress and controlling antioxidant enzymes through the Nrf2/HO–1 and NQO1 pathways, it improves cellular function by lowering protein–related apoptosis.

CONCLUSIONS

In conclusion, propolis treatment substantially increase glucose consumption by enhancing the activities of PK and SOD. These results enhanced the likelihood that propolis might be used as an adjuvant therapy to shield organs from the oxidative stress caused by DOX. Molecular docking studies reported the interaction mode of some propolis components (CAPE and Quercetin) and DOX with PK and SOD including some H–bonds, metal coordination and close contact interactions, similarly to current cocrystals, in previously represented binding sites. Especially when the interaction types and docking scores obtained on PK are examined, it can be said that CAPE and Quercetin show higher affinity than DOX. Studies have revealed that CAPE and Quercein will inhibit both PK and SOD at an estimated micromolar (μM) level. To confirm the significance of propolis in the curative management of DOX–induced multiple toxicities or to develop new drug delivery techniques, more research is required.

Conflict of interest

There are no conflicts of interest, according to the authors, regarding this article.

Credit author statement

Seval Yilmaz: This author was involved in the design of the study, animal applications, laboratory experiments, and evaluation of results.

Emre Kaya: This author was involved in the animal applications, laboratory experiments, and evaluation of results.

Harun Yonar: This author supported the statistical evaluation of the results of the study.

Harun Uslu: This author took part in the molecular docking analyzes in the study.

Ethics approval and consent to participate

The Firat University Animal Studies Local Ethics Committee accepted the experiments (Protocol No: 2012/03-43), which were carried out strictly in compliance with the Experimental Animal Ethics Committee's Guiding Principles.

Disclosure statement

The authors state that there are no interests at odds with one another.

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