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BIOCHEMICAL INVESTIGATION OF VITAMIN AND PHYTOSTEROL CHANGES IN PISTACHIO EXTRACT IN *SACCHAROMYCES CEREVISIAE*

Zehra GÖKÇE [1]

[1] *Kilis 7 Aralık University, Department of Nutrition and Dietetics, Kilis, Türkiye, zhrgkc23@gmail.com*

ABSTRACT

In this study, the effects of *Pistacia vera* (pistachio, PV) extract grown in Kilis province on vitamin and phytosterol profiles in *Saccharomyces cerevisiae* under carbon tetrachloride (CCl_4)-induced oxidative stress were investigated. *S. cerevisiae* FMC16 was used as a eukaryotic cell culture model and cultivated in YEDP medium containing yeast extract, bactopeptone, and glucose. Oxidative stress was induced by CCl_4 to evaluate the protective potential of pistachio extract against oxidative damage.

Vitamin and phytosterol compositions of *P. vera* fruit extract were determined using high-performance liquid chromatography (HPLC). The phytochemical characterization of the extract revealed a total phenolic content (TPC) of 116.94 ± 0.27 µg gallic acid equivalents (GAE)/g and a total proanthocyanidin (TP) content of 5.41 mg catechin equivalents (CE)/g, indicating strong antioxidant capacity. Six experimental groups were designed: control, CCl_4, PV 200 µl (PV2), PV 400 µl (PV4), PV2 + CCl_4, and PV4 + CCl_4. Following sterilization, PV extract and CCl_4 were added to yeast cultures, which were incubated at 30 °C for 1, 3, 5, and 72 hours. Cell growth parameters, vitamin levels, and phytosterol contents in yeast cells were subsequently analyzed by HPLC.

CCl_4 exposure caused significant reductions in several vitamins and sterols in *S. cerevisiae*, indicating pronounced oxidative stress. Ergosterol levels, which are essential for yeast cell membrane integrity, were significantly decreased in the CCl_4-treated group compared to the control. In contrast, *Pistacia vera* extract attenuated these reductions and improved antioxidant defense responses in yeast cells. Overall, pistachio extract exhibited protective effects against oxidative damage in *S. cerevisiae*. These findings suggest that *P. vera* may serve as a valuable natural antioxidant source for future network-based pharmacology and bioinformatics studies.

Keywords: *Pistacia vera, Saccharomyces cerevisiae*, Oxidative stress, Vitamins, Phytosterols.

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1 INTRODUCTION

Pistachios and hazelnuts are among the most consumed nuts worldwide [1]. Although the term “nut” is currently used to describe various plant species and their fruits, it is primarily associated with the edible fruits of only one species within the *Pistacia* genus *Pistacia vera* by consumers. *Pistacia vera* is marketed globally [2] and is considered a key economic crop in several countries. The leading consumers of pistachios include China, the USA, Türkiye, Iran, Germany, Syria, Spain, Vietnam, Italy, India, France, and Afghanistan, which together account for approximately 71.40% of global pistachio consumption [3].

Current scientific evidence suggests that pistachios, among all tree nuts, contain the highest amount of gamma-tocopherol, as well as other bioactive compounds such as beta-carotene, lutein, and zeaxanthin [4]. Due to these properties, natural *Pistacia vera* L. (PvL) is regarded as a potent antioxidant-rich food. Since antioxidants are widely used in the treatment of free radical-induced diseases, the identification of new natural antioxidant sources is of significant importance [5]. However, from a metabolic perspective, sterols play a significant role in energy balance and membrane transport capacity. Considering that yeast cells require exogenous sterols for anaerobic trafficking, phytosterols such as β-sitosterol characterize growth and metabolic balance [6]. In organisms such as Saccharomyces cerevisiae, where ergosterol is the predominant sterol, exogenous phytosterols can perform a specific function of ergosterol [7]. Phytosterols derived from pistachios may play important roles in yeast biochemistry and may also play a protective role against reactive oxygen species (ROS) in yeast degradable cells.

Reactive oxygen species (ROS) cause damage to cellular macromolecules, resulting in lipid peroxidation and alterations to nucleic acids and proteins (REF). Antioxidant defense systems halt oxidative damage; however, when these systems are inadequate, oxidative damage occurs within the cell (REF). Oxidative stress arises when intracellular antioxidant defense mechanisms fail to eliminate ROS completely [8]. Several factors, such as exposure to xenobiotics and ionizing radiation, promote oxidative stress and the formation of free radicals.

Carbon tetrachloride (CCl_4) is used in the synthesis of chlorofluorocarbons, in cleaning agents, as a solvent, and in grain sanitation. It is an intermediate that accumulates degradation products within the cell, potentially leading to cellular damage [9], [10]. When oxidative stress becomes severe, it can cause denaturation and degradation of proteins and lysosomal membranes, ultimately resulting in cell death [11].

Given its prominence as a cellular model, *Saccharomyces cerevisiae* was employed in this study [9]. This yeast species is frequently used in xenobiotic, toxicological, biochemical, and molecular research due to its resistance to various hazardous chemicals. Such resistance can manifest through decreased metal uptake, limited metal transport, or intracellular metal sequestration. Although some studies have demonstrated metabolic differences in yeast cells, their metabolic responses and behavior towards xenobiotics closely resemble those of higher organisms [9]-[12].

This study aimed to biochemically investigate the potential protective effects of *Pistacia vera* (PV) extract, cultivated in Kilis (Türkiye), against CCl_4-induced cellular damage in *Saccharomyces cerevisiae*, specifically focusing on its impact on vitamin and phytosterol parameters.

2 MATERIAL AND METHOD

2.1 Materials

Saccharomyces cerevisiae FMC16. (baker's yeast) was used as the cell culture model. Fresh *Pistacia vera* fruits were obtained from Bozkaya Village, located in the Musabeyli district of Kilis, Türkiye.

2.2 Preparation of Plant Extracts

Plant samples were obtained from the Musabeyli district of Kilis. Fifty grams of pistachio nuts were homogenized in a blender with 50 mL of methanol for 10 minutes. The same process was repeated using Ethanol as a solvent. And hexane is the solvent used to obtain oils and other non-polar compounds in the sample, methanol is the solvent used to obtain phenolic compounds and polar compounds. The resulting homogenates were combined, and the solvents were evaporated using a rotary evaporator (Buchi Rotavapor R-210 model) to obtain the extract. Glass bottles autoclaved at 120°C for 15 minutes and total of 200 mL of extract was transferred into 250 mL glass bottles. Each sample was prepared in five replicates under identical conditions. Once the extracts cooled to room temperature, 1 mL of yeast was added to each and incubated at 30°C.

2.3 Preparation of Yeast Culture and Cell Growth

To support the growth and proliferation of *S. cerevisiae* FMC 16 , a YEDP (for 100 mL: 1 g yeast extract, 2 g bactopeptone, 2 g glucose) was prepared [13]. The experimental groups were as follows:

1. **Control group (n=5):** Only YEDP was added.
2. **CCl_4 group (C) (n=5):** YEDP and 100 µL of 99.5% pure CCl_4 chemical were added at the 24th hour of incubation.
3. **Pistachio group (PV2) (n=5):** YEDP and 200 µL of pistachio extract were added.
4. **Pistachio group (PV4) (n=5):** YEDP and 400 µL of pistachio extract were added.
5. **Pistachio + CCl_4 group (PV2C) (n=5):** YEDP and 200 µL of pistachio extract added, with 100 µL of 99.5% pure CCl_4 chemical were added at the 24th hour of incubation.
6. **Pistachio + CCl_4 group (PV4C) (n=5):** YEDP and 400 µL of pistachio extract added, with 100 µL of 99.5% pure CCl_4 chemical were added at the 24th hour of incubation.

After treatment, all samples were incubated for 72 hours. Cell density in the culture medium was measured at 600 nm using a spectrophotometer. At the end of the 72-hour incubation period, 4% BHT (butylated hydroxytoluene) was added to the samples in each group to prevent further oxidation. Following this, cells from each culture were transferred into sterile Falcon tubes, centrifuged at 6000 rpm at 4°C, and the pellets were collected. The pellets were washed with 0.9% NaCl, and their wet weights were determined. The pellets were then homogenized using phosphate and Tris buffer, followed by another homogenization step with a hexane/isopropyl alcohol mixture (3:2, v/v). Lipophilic molecules, including vitamins, phytosterols, and alpha-tocopherol, were then measured (REF).

2.4 HPLC Analysis of Fat-Soluble Vitamins (A, D, E, K), Ergosterol, and Phytosterols

Cell pellets were homogenized in a hexane/isopropyl alcohol mixture (3:2, v/v) and centrifuged. The supernatant was collected into 25 mL capped tubes, to which 5% KOH solution was added. After vortexing, the tubes were incubated at 85°C for 15 minutes, then cooled to room temperature. Five mL of distilled water was added, and non-saponified lipophilic compounds were extracted twice with 5 mL of hexane. The solvent was evaporated under

nitrogen gas, and the residue was dissolved in 1 mL of an acetonitrile/methanol (50:50, v/v) mixture. The samples were placed in autosampler vials for HPLC analysis (REF).

The mobile phase consisted of acetonitrile/methanol (60:40, v/v) at a flow rate of 1 mL/min. Detection was performed using a PDA-UV detector with a Supelcosil LC-18 column (15 × 4.6 cm, 5 µm; Sigma, USA). Analysis of vitamin E, other fat-soluble vitamins, and phytosterols was conducted at 202 nm [14]–[16].

2.5 Phytochemical Compounds

2.5.1 Total phenolic contents (TPC)

Total phenolic contents (TPC) was determined using the method outlined by Slinkard and Singleto [17]. The gallic acid (GAE) was used as a standard. The recorded absorbances were calculated as the total amount of phenolic compounds contained in the plant extract as gallic acid equivalent (mg gallic acid/g extract).

2.5.2 Total proanthocyanidin content (TP)

Total proanthocyanidin content (TP) was calculated according to the approach published by Amaeze et al. [18]. The catechin was used as a standard.

2.6 Evaluation of Experimental Data

Data were analyzed using SPSS 15.0 statistical software. Differences between the control and treatment groups were first evaluated using one-way ANOVA, followed by the LSD test to determine specific group differences. Results were presented in tables and graphs as mean ± standard error of the mean (mean ± SEM). Statistically significant differences were indicated using distinct letters in the tables, and significance levels were expressed in the text as $p > 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.001$.

3 RESULTS AND DISCUSSION

In the methanolic extract of *P. vera* L., the presence of tocopherols (Σ-tocopherol, α-tocopherol, β-tocopherol, ɣ-tocopherol, δ-tocopherol), vitamins K1, K2, D2, D3, A1, as well as phytosterols such as campesterol, ergosterol, stigmasterol, and β-sitosterol, was determined by HPLC analysis (Table 1, Figure 1).

Table 1. Lipophilic vitamin and phytosterol content of Pistacia vera (µg/g).

Lipophilic Vitamins, Phytosterols	*Pistacia vera*
Σ-Tocopherol	1.753
α-Tocopherol	11.92
β-Tocopherol	0.37
ɣ-Tocopherol	20.46
δ-Tocopherol	0.85
Vitamin K1	19.81
Vitamin K2	3.49
Vitamin D2	39.35
Vitamin D3	15.92
Retinol (Vitamin A1)	0.06
Campesterol	1.24
Ergosterol	11.5
Stigmasterol	342.7
β-sitosterol	325.8

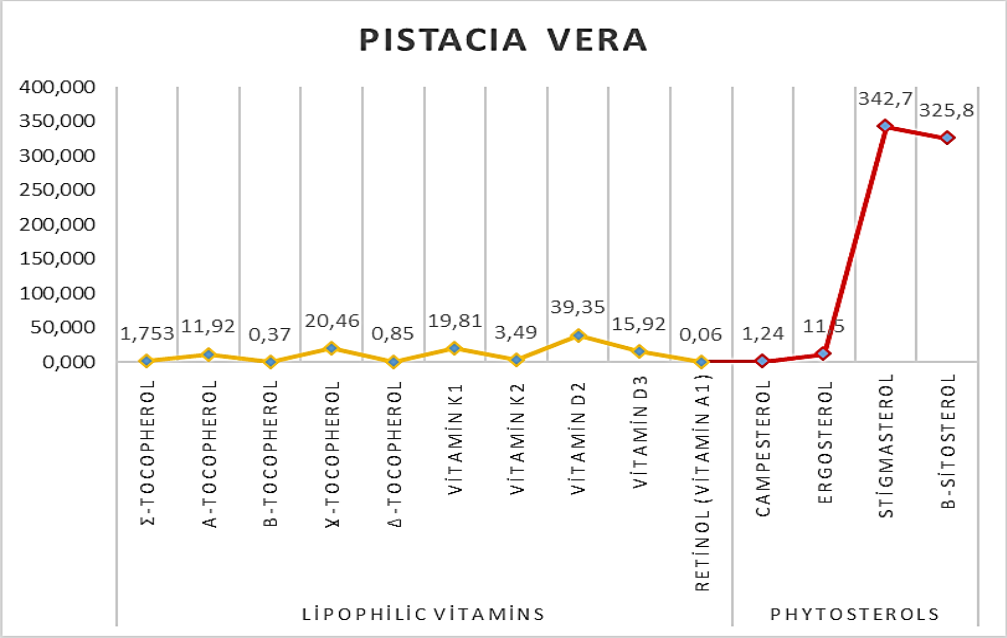


Figure 1. Lipophilic vitamin and phytosterol content of Pistacia vera (µg/g).

Table 2 shows the amount of total phenolic compounds (TPC), total proanthocyanidin (TP) in *P. vera* extract. TPC amounts was determined 116.94±0.27 µg GAE/g and TP amounts was 5.41 mg CE/g, extract *P. vera* extract.

Table 2. Total phenolic, and total proanthocyanidin contents of Pistacea vera extracts.

	Total phenolic *(TPC)*	**Total proanthocyanidin *(TP)***
Pistacea vera	116.94±0.27 µg GAE/g	5.41 mg CE/g

Total phenolic compounds and proanthocyanidins in pistachios are important biomolecules that reduce oxidative stress, regulate metabolic processes, and influence cell development in both yeast and mammalian cells. Phenolics can accelerate the transition to S phase and delay aging by affecting cell cycle proteins. A study by Farrall et al. [19] reported that polyphenol application increased expression and promoted proliferation in yeast cells. A study on metabolic regulation reported increased mitochondrial activity and glucose utilization in yeast cells treated with proanthocyanidins. Proanthocyanidins may enhance energy metabolism [20]. Another study demonstrated that *P. vera* polyphenols reduced ROS levels and increased proliferation in fibroblast cells, highlighting their antioxidant and cell-regenerating effects [21]. In our research findings, it was determined that pistachio extract increased cell growth depending on the amount of total phenolic compounds and proanthocyanidins and reduced the level of ROS caused by CCl_4.

3.1 Effect of *Pistacia vera* Extracts and CCl_4 on Vitamin and Phytosterol Profiles in *S. cerevisiae* Culture

Compared to the control group, a statistically significant decrease in vitamin K1 and K2 levels was observed in the CCl_4 group ($p<0.01$). The PV2 and PV4 groups showed a partial and considerable increase, respectively, in vitamin K1 and K2 levels compared to the control ($p<0.05$; $p<0.01$). Moreover, PV2+CCl_4 and PV4+CCl_4 groups exhibited partial and significant increases, respectively ($p<0.05$; $p<0.01$) (Table 3).

Vitamin D2 levels significantly decreased in the CCl_4 group ($p<0.001$), while PV2 and PV2+CCl_4 showed moderate increases, and PV4 and PV4+CCl_4 exhibited a highly significant increase ($p<0.001$; $p<0.05$; $p<0.001$). For vitamin D3, no significant difference was found in the CCl_4 group, but the other groups showed slight increases ($p>0.05$; $p<0.05$) (Table 3).

δ-Tocopherol levels were moderately elevated in the CCl_4 group, while the PV2+CCl_4 and PV4+CCl_4 groups showed significant increases compared to control ($p<0.05$; $p<0.01$). PV2 and PV4 groups also demonstrated highly significant increases ($p<0.001$). Regarding α-tocopherol, while the CCl_4 group showed no significant change, PV2+CCl_4 and PV4+CCl_4 had moderate increases ($p>0.05$; $p<0.05$), and PV2 with PV4 groups showed statistically significant increases ($p<0.01$). Σ-tocopherol levels increased moderately in the CCl_4 group, significantly in PV2 and PV2+CCl_4 groups, and highly significantly in PV4 and PV4+CCl_4 groups ($p<0.05$; $p<0.01$; $p<0.001$) (Table 3).

Retinol levels significantly decreased in the CCl_4 group compared to control ($p<0.001$). PV2 and PV4 groups showed moderate increases, while PV2+CCl_4 and PV4+CCl_4 groups exhibited significant increases ($p<0.05$; $p<0.01$). Retinol acetate levels showed no significant difference in the CCl_4 group, while moderate increases were detected in the other groups ($p>0.05$; $p<0.05$) (Table 3).

Regarding the changes in phytosterols, no significant difference was observed in the retinol acetate level in the CCl_4 group. And a statistically significant increase was observed in the PV2, PV4, PV2+CCl_4 and PV4+CCl_4 groups compared to the control ($p<0.05$; $p<0.01$). In terms of ergosterol levels, a significant decrease was observed in the CCl4 group and a significant increase in the PV2 and PV4 groups compared to the control group ($p<0.001$). β-Sitosterol and Stigmasterol levels were significantly increased in the PV2+CCl_4 and PV4+CCl_4 groups compared to the control group ($p<0.001$) (Table 3).

Table 3. Effect of pistachio extracts and CCl_4 on the vitamin and phytosterol profile of S. cerevisiae yeast cell (µg/1g)*.

Vitamins and Phytosterols	Control	CCl_4	PV2	PV4	PV2+CCl_4	PV4+CCl_4
Vitamin K1	1.95 ± 0.21^{a}	1.23 ± 0.11^{c}	2.54 ± 0.20^{b}	2.80 ± 0.16^{c}	2.40 ± 0.13^{b}	2.68 ± 0.09^{c}
Vitamin K2	5.64 ± 0.06^{a}	4.06 ± 0.03^{c}	6.15 ± 0.06^{b}	6.97 ± 0.09^{c}	6.28 ± 0.09^{b}	6.39 ± 0.16^{c}
Vitamin D2	2.17 ± 0.03^{a}	0.63 ± 0.04^{c}	3.06 ± 0.05^{b}	3.62 ± 0.06^{c}	2.84 ± 0.02^{b}	3.07 ± 0.06^{c}
Vitamin D3	1.05 ± 0.04^{a}	1.14 ± 0.07^{a}	1.22 ± 0.03^{b}	1.25 ± 0.02^{b}	1.26 ± 0.11^{b}	1.28 ± 0.10^{b}
δ-Tocopherol	21.46 ± 3.13^{a}	18.41 ± 0.03^{b}	42.86 ± 0.25^{d}	47.87 ± 0.32^{d}	35.64 ± 0.01^{c}	38.25 ± 0.01^{c}
α-Tocopherol	29.29 ± 0.22^{a}	32.21 ± 0.20^{a}	40.06 ± 0.27^{c}	42.87 ± 0.36^{c}	37.49 ± 0.51^{b}	38.81 ± 0.28^{b}
Σ-Tocopherol	0.25 ± 0.07^{a}	0.41 ± 0.07^{b}	1.24 ± 0.08^{c}	1.40 ± 0.07^{d}	1.16 ± 0.17^{c}	1.91 ± 0.06^{d}
Retinol (Vitamin A1)	6.44 ± 0.07^{a}	2.05 ± 0.06^{d}	7.31 ± 0.06^{b}	8.46 ± 0.07^{b}	4.05 ± 0.10^{c}	4.75 ± 0.06^{c}
Retinol acetate	0.18 ± 0.06^{a}	0.19 ± 0.11^{a}	0.29 ± 0.01^{b}	0.33 ± 0.05^{b}	0.21 ± 0.16^{b}	0.24 ± 0.07^{b}
Ergosterol	19.28 ± 0.50^{a}	10.39 ± 0.23^{d}	27.94 ± 0.38^{d}	29.98 ± 0.17^{d}	21.61 ± 0.56^{b}	24.91 ± 0.21^{c}
β-Sitosterol	97.77 ± 2.81^{a}	14.67 ± 0.01^{d}	98.15 ± 0.05^{a}	112.66 ± 0.26^{b}	67.61 ± 0.17^{c}	78.93 ± 0.54^{c}
Stigmasterol	119.93 ± 1.12^{a}	148.55 ± 2.67^{b}	274.84 ± 3.21^{d}	296.06 ± 3.23^{d}	218.06 ± 1.65^{c}	231.46 ± 1.66^{c}

*The meaning of the symbols is given below.

*Each value is the mean±S.E. (standard error) of 5 repetitions. Superscripts after values in the same line with different letters represent a significant difference. a: $p>0.05$, b: $p<0.05$, c: $p<0.01$, d: $p<0.001$

a: Values of $p>0.05$ is not statistically significant b: Values of $p<0.05$ is statistically significant

c: Values of $p<0.01$ is statistically more significant d: Values of $p<0.001$ is statistically most significant

CCl_4: Carbontetrachloride (100 µl). **PV2:** *Pistacia vera* (200 µl). **PV4:** *Pistacia vera* (400 µl).

PV2+CCl_4: *Pistacia vera* (200 µl) +Carbontetrachloride (100 µl).

PV4+CCl_4: *Pistacia vera* (400 µl) +Carbontetrachloride (100 µl).

Fatty acid profiles are not the sole determinants of the nutritional quality of nuts; micronutrients and compounds such as tocopherols also contribute significantly. Due to its high content of protein, fats, fatty acids, vitamins, minerals, and antioxidants, *P. vera* possesses considerable nutritional value [22]. These characteristics suggest beneficial health effects of pistachios.

Pistachios are a rich source of lipid-soluble antioxidants (vitamins A, D, E, K), which are known to help regulate oxidative stress and reduce the risk of various chronic diseases [23]. Mandalari et al. also reported that pistachios contain high levels of vitamins A, D, E, and K compared to other foods [24]. In parallel with these studies, our findings confirmed that *P. vera* L. methanolic extract contains significant levels of tocopherols and fat-soluble vitamins, as well as phytosterols such as campesterol, ergosterol, stigmasterol, and β-sitosterol (Table 1, Figure 1). Our study further demonstrated that induction of oxidative stress with CCl_4 in yeast cells significantly altered levels of vitamins and sterols (Table 3). Under anaerobic conditions, yeast cells are highly dependent on exogenous sterols. Phytosterols such as β-sitosterol are crucial in this regard. Our results, showing elevated β-sitosterol levels in pistachio-containing groups, are consistent with other studies [25]. The differing doses of PV extract had varying impacts on vitamin levels, both in quantity and significance of change (Table 3).

In this study, CCl_4 administration was found to reduce the levels of several vitamins in *S. cerevisiae*. Conversely, PV extract supplementation at different concentrations led to significant increases in vitamin levels compared to the CCl_4 group (Table 3). Consistent with our results, a previous study also reported that PV extract mitigated CCl_4-induced oxidative damage in *S. cerevisiae* [9]. Furthermore, Ciudad-Mulero et al. investigated the role of various nuts, including pistachios, in human clinical trials and found that they significantly reduced oxidative stress. As oxidative stress biomarkers are closely linked to pathological disorders such as cardiovascular and neurodegenerative diseases, dietary habits play a vital role in maintaining health [25]. Canudas et al. showed that a pistachio-enriched diet reduced DNA damage caused by oxidative stress in 49 prediabetic individuals [26].

Plant sterols are found in low concentrations in vegetable oils, nuts, seeds, whole grains, and vegetables; however, supplements are often required for therapeutic effects. Plant sterols are lipophilic compounds naturally found in plants and structurally similar to animal cholesterol. Current evidence suggests that a daily intake of approximately 2 g of plant sterols/stanols reduces total cholesterol, and particularly LDL-cholesterol, by an average of 7–

12% [27]. Some meta-analyses, such as the study by Zhang et al. [28] reported that dietary plant sterols reduce total cholesterol and LDL-cholesterol levels while increasing HDL-cholesterol.

Our results are parallel with previous studies CCl_4 application in *S. cerevisiae* significantly reduced levels of phytosterols (stigmasterol, ergosterol, β-sitosterol) compared to control. Ergosterol, essential for yeast cells, showed a significant reduction in the CCl_4 group, while PV2, PV4, PV2+CCl_4, and PV4+CCl_4 groups showed marked increases, especially in PV2 and PV4 (Table 3). This suggests that PV extract may enhance ergosterol biosynthesis, potentially improving fermentation capacity in *S. cerevisiae*. Ergosterol is the primary sterol of the cell membrane in all yeast species, particularly *Saccharomyces cerevisiae*, and plays a critical regulatory role in membrane fluidity, permeability, and stability. Increased ergosterol levels strengthen the physicochemical structure of the membrane, enabling the yeast cell to better tolerate both environmental stresses and the metabolic loads imposed by fermentation conditions [29]. By regulating the positioning of membrane proteins, ergosterol enables critical processes such as nutrient uptake, ion pumping, and signal transduction to function more effectively under stress conditions [30]. Increased ergosterol levels may provide versatile biological advantages, such as preserving yeast cell membrane integrity, optimizing fermentation capacity, and increasing tolerance to toxic substances and environmental stresses.

The protective role of pistachios against CCl_4-like toxicity in animal models was investigated in rat studies by Ghorbani et al. [31] The effects against liver damage showed that *P. vera* extract significantly increased cellular antioxidant capacity in hemolytic and oxidative damage models. These findings are thought to be related to the antioxidant defense-supporting content (vitamins, phytosterols, and phenolics) of PV extract in the S. cerevisiae model used in our study (Table 1, Figure 1). In this context, PV extract added to the CCl_4 group, which is our toxicant group, statistically significantly increased the levels of vitamins K1, K2, and D3, as well as the levels of phytosterols ergosterol, β-sitosterol, and stigmasterol (Table 3). Our study results are consistent with previous studies. Furthermore, this protective effect detected in yeast cells confirms this at the organismal level. The ergosterol reduction observed in our S. cerevisiae model and the antioxidant protective effects of PV extract are consistent with the effects observed against CCl_4 toxicity in animal (especially rat) models. This parallelism suggests that our yeast model could play a leading role in understanding CCl_4 toxicity pathways and protective molecules and provide a strong basis for generating hypotheses for future animal/human studies.

In recent years, researchers have increasingly utilized extracts from natural products and applied advanced techniques [32], Particularly in network-based pharmacological studies, phytochemicals from vitamin- and fatty acid-rich plants have been used [33], [34]. Kumar et al. investigated interactions between phytochemicals and various proteins to identify potential drug targets for diseases such as cancer, diabetes, neurodegeneration, and cardiovascular conditions [35]. Several studies have explored the bioavailability of natural products [36]–[39]. Our study on *S. cerevisiae* using vitamin- and sterol-rich *Pistacia vera* suggests potential applications in more advanced informatics-based nutritional and pharmacological research.

4 CONCLUSION AND SUGGESTIONS

In this study, which investigated the effects of carbon tetrachloride (CCl_4) on the vitamin and phytosterol contents in *Saccharomyces cerevisiae*, it was concluded that CCl_4 poses a significant risk to cell viability. The use of *Pistacia vera* (PV) extract to counteract the oxidative damage induced by CCl_4 demonstrated beneficial effects on both vitamin and phytosterol levels. These effects may be attributed to the interaction of plant extracts and chemical agents with vitamin and phytosterol metabolism in yeast cells. However, to date, no prior study has specifically examined the biochemical effects of *Pistacia vera* extract on *S. cerevisiae* cells exposed to CCl_4.

S. cerevisiae is a eukaryotic model organism widely used in scientific research due to its similarity to human metabolic processes. Studies conducted using *S. cerevisiae* often serve as models for understanding similar phenomena that may occur in the human body. Based on the findings, it can be inferred that xenobiotics—substances that accumulate in cells and organs and exhibit long-term toxic effects—have a detrimental impact on vitamin and phytosterol content. Furthermore, the extent of these effects appears to vary depending on the concentration of the xenobiotic applied.

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Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

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