

# The Effect of *Pistacia vera* L. (Pistachio) Extract on Fatty Acid Biosynthesis and Total Proteins against Carbon Tetrachloride (CCl<sub>4</sub>)-induced Damage in *Saccharomyces cerevisiae*

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## ABSTRACT

**Background and Aim:** *Pistacia vera* (PV) is an important species with economic value, rich compound content and high biological activity. Carbon Tetrachloride (CCl<sub>4</sub>) used in our study is an important reactive toxic compound. *Saccharomyces cerevisiae* is often used as an important cell model in xenobiotic, toxicological and biochemical studies. This research, fatty acid contents of *Pistacia vera* cultivated in Kilis province, the effects of this content on fatty acid profile and total proteins in CCl<sub>4</sub>-induced cell damage the *Saccharomyces cerevisiae* (bread yeast) were investigated. **Materials and Methods:** Fatty acid analysis of pistachio fruits was performed with GC tools. The model used for cell culture was *S. cerevisiae*. YEDP (1 g yeast extract for 100 mL, 2 g bacto peptone, 2 g glucose) medium was utilized for the growth and multiplication of *S. cerevisiae* FMC16. There were six groups in this study. i) Control group, ii) *Pistacia vera* 200 µL (PV2) group; iii) Carbon Tetrachloride 100 µL (CCl<sub>4</sub>) group, iv) *Pistacia vera* 400 µL (PV4) group; v) PV2+CCl<sub>4</sub> group; and vi) PV4+CCl<sub>4</sub> group. Following sterilization, *S. cerevisiae* cultures were incubated at 60°C for 72 hr (overnight). PV and CCl<sub>4</sub> were then added to the cultures. Cell growth and total protein amounts of *S. cerevisiae* were determined by spectrophotometer. **Results:** Research of results showed that, in contrast to the CCl<sub>4</sub> group, total protein synthesis and cell proliferation increased in PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups at 1, 3, 5 and 72 hr (overnight). In our study, it was determined that there were important changes in fatty acids profile levels of oxidative stress-induced yeast cells. In our findings, an increase was observed in some fatty acid levels of PV extract, PV2, PV4, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups compared to CCl<sub>4</sub> groups. In our study, decreasing effects of CCl<sub>4</sub> treatment on many fatty acids were observed in *S. cerevisiae*. **Conclusion:** PV extract helps *S. cerevisiae* culture cells thrive and synthesize all of their proteins while also lowering oxidative damage. The positive results of PV extract especially on fatty acid biosynthesis and responsible enzyme activities will be a source for analogous research on different living organisms.

**Keywords:** *Pistacia vera*, CCl<sub>4</sub>, *Saccharomyces cerevisiae*, Fatty acids, Total protein.

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## INTRODUCTION

Nutrient-dense foods like pistachio (*Pistacia vera* L.) nuts have a distinctive profile of high-quality protein, minerals, lipids, antioxidants, and vitamins.<sup>1</sup> In addition to being a great source of numerous vital bioactive substances, pistachios have a number of positive health effects on people.<sup>2</sup> *Pistacia vera* (PV) has a variety of bioactive substances that are highly effective in reducing the risk of chronic diseases and controlling oxidative stress.<sup>3,4</sup> Protein, fat, and glucose rates can be impacted by Reactive Oxygen Species

(ROS) and nucleic acids. Antioxidant defense stops oxidative damage. However, oxidative damage happens in the cell when the antioxidant defense mechanism is insufficient. When cellular antioxidant defense mechanisms fail to completely remove ROS, oxidative stress results.<sup>5</sup>

Numerous factors, such as the harmful effects of xenobiotics or ionizing radiation, promote oxidative stress and the formation of free radicals.<sup>6</sup> In the synthesis of chlorofluorocarbons, the creation of cleaners and solvents, as well as the sanitation of cereals, all include the use of Carbon Tetrachloride (CCl<sub>4</sub>) as an intermediate product. It is an environmental toxin that results in reactive oxygen species in the living system, which leads to oxidative stress. The metabolic activation of short-lived reagent intermediate products and the toxic impact of CCl<sub>4</sub> are strongly



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connected.<sup>7</sup> The accumulation of fragmentation products inside the cell causes additional harm. This is the harmful effect of CCl<sub>4</sub>. When oxidative stress becomes severe, it can cause the degradation of denaturation of proteins, lysosome membranes, and cell death.<sup>8</sup> Because *Saccharomyces cerevisiae* occurs in the most significant cell model, *S. cerevisiae* will be chosen in our investigation for these reasons.<sup>6,9,10</sup> Typically, xenobiotic, toxicological, biochemical, and molecular research use this cell module. Because of their diverse characteristics, yeasts exhibit resistance to hazardous chemicals. This resistance may manifest itself through a variety of methods, including metal intake, a decrease in metal transit, or metal retention in cells. Although these yeast cells' metabolic characteristics differ in certain ways, many of their traits have been shown to be similar to those of advanced organisms, including parallelism in metabolic reactions and the presence of xenobiotics.<sup>6,11,12</sup>

The aim of this study is to examine the effects of adding PV extract and CCl<sub>4</sub> to the *S. cerevisiae* growth medium using pistachios fruit grown in the Kilis region on some biochemical parameters such as total protein measurements, cell concentration measurements and fatty acid measurements.

## MATERIALS AND METHODS

### Herbal materials

PV fruit from Musabeyli in Kilis, Turkey, was employed as a source of herbal ingredients. PV fruit was removed using 85% methanol.

### The research group and growth factors

Six media groups were created in six parallels, including the control groups, the CCl<sub>4</sub> (100 µL) groups, the *Pistacia vera* 200 µL (PV2) groups, the *Pistacia vera* 400 µL (PV4) groups, the *Pistacia vera* 200 µL (PV2)+CCl<sub>4</sub> groups, and the *Pistacia vera* 400 µL (PV2)+CCl<sub>4</sub> groups. After sterilization, *S. cerevisiae* cultures were given injections of PV and CCl<sub>4</sub>, and the cultures were allowed to grow for 1, 3, 5, and 72 hr (overnight) at 30°C.<sup>13</sup> These samples were centrifuged for 5 min at 4°C at 5000 rpm in 5 min after incubation. After being acquired by centrifugation, the pellets were weighed. The solution of 50 mM KH<sub>2</sub>PO<sub>4</sub> was used to wash the pellets. The buffer solution, consisting of 50 mM Tris and 20 mM EDTA, was prepared in 10 mL of a pH of 7.40. The pellets were homogenized in the cold medium. After the procedure, these samples were centrifuged for 5 min at 9000 rpm to cool them. The pellets were separated for fatty acid and phytosterol analysis. Total protein was analyzed in the supernatants.

### Total protein measurements

Total protein contents of yeast cells were determined according to<sup>14</sup> by using BSA (Bovine Serum Albumin) as a standart. The absorbance was read using a spectrophotometer at 600 nm.

## Gas chromatography for fatty acid isolation

By combining 10 mL of n-hexane and isopropyl at a 3/2 (v/v) ratio, samples were homogenized. By adding 10 mL of a 3/2 (v/v) hexane/isopropanol combination<sup>15</sup> to the liquid phases of these samples that remained after LPO, fatty acids were separated. Then, hexane phase was introduced to several test tubes, followed by the addition to 2% methanolic sulfuric acid at 5 mL. The combination was then incubated in the incubator for 12 hr at 55°C. Thus, the tube was filled with 5% sodium chloride, followed by the extraction of fatty acids methyl ester into pure hexane of 5 mL. Using a nitrogen stream, the hexane phase was evaporated from 5 milliliters of a 2% KHCO<sub>3</sub> solution.<sup>16</sup> Finally, 1 mL of heptane was used to dissolve the fatty acids methyl esters residues before they were put into vials. A device called the Shimadzu GC 17 was the methyl ester of fatty acids measured in Kyoto, Japan.

## Cell concentration measurements

The culture samples used in these tests were grown at 30°C for 1, 3, 5 hr, and overnight (72 hr). The measurement has been carried out using a spectrophotometer at 600 nm (OD<sub>600</sub>).<sup>17</sup>

## Data analysis

The software SPSS 15.0 was used to evaluate the findings. The comparison of the controls and experimental group using the ANOVA approach with LSD testing. The obtained data were reported as mean ±SEM. The statistical important of material was determined by comparing the group differences,  $p > 0.05$ ,  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  value.

## RESULTS

When *S. cerevisiae* was compared with control and CCl<sub>4</sub> groups in terms of different developmental times at 3 hr, 5 hr and 72 hr, cell growth was statistically significantly decreased in the CCl<sub>4</sub> groups ( $p < 0.05$ ) (Table 1, Figure 1). Additionally, it has been seen that in common PV provided groups had increased cell density in comparison to the control when analysis results from taking 1 hr, 2 hr, and 4 hr measurements of cell densities are evaluated (Table 1, Figure 1). When the development of *S. cerevisiae* was compared in terms of 3 hr and 5 hr, a statistically important increase was observed in the PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> group contrary to control groups ( $p < 0.01$ ) (Table 1, Figure 1).

When the effect of control group and groups given pistachio extract on total protein amount in *S. cerevisiae* was examined. It was determined that the increase in PV2 and PV4 groups was statistically important in correlation with the group according to control ( $p < 0.01$ ). When the CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> group were compared with control groups in terms of total protein amount, a partial decrease in the CCl<sub>4</sub> group and an increase in the partial levels in the others were found statistically ( $p < 0.05$ ) (Figure 2). As a results of Figure 2 is analyzed; large protein quantity has been calculated in PV2 (200 µL), PV4 (400

**Table 1: 1, 3, 5 hr and overnight (72 hr) *S. cerevisiae* cell growth density amounts for improving (OD<sub>600</sub> 30°C).**

Groups	1 hr	3 hr	5 hr	Overnight
Control	1.73±0.03 <sup>a</sup>	1.76±0.00 <sup>a</sup>	1.75±0.00 <sup>a</sup>	1.98±0.06 <sup>a</sup>
CCl <sub>4</sub>	1.71±0.01 <sup>a</sup>	1.73±0.00 <sup>b</sup>	1.72±0.00 <sup>b</sup>	1.67±0.00 <sup>b</sup>
PV2	1.86±0.00 <sup>b</sup>	1.88±0.00 <sup>c</sup>	1.89±0.00 <sup>c</sup>	1.92±0.00 <sup>a</sup>
PV4	1.87±0.00 <sup>b</sup>	1.90±0.00 <sup>b</sup>	1.91±0.00 <sup>c</sup>	1.95 ±0.02 <sup>b</sup>
PV2 +CCl <sub>4</sub>	2.25±0.00 <sup>c</sup>	2.38±0.00 <sup>c</sup>	2.29±0.01 <sup>c</sup>	2.07±0.01 <sup>b</sup>
PV4+ CCl <sub>4</sub>	2.51±0.01 <sup>c</sup>	2.34±0.01 <sup>c</sup>	2.38±0.01 <sup>c</sup>	2.10±0.04 <sup>b</sup>

CCl<sub>4</sub>: Carbon tetrachloride (100 µL), PV2: *Pistacia vera* (200 µL), PV4: *Pistacia vera* (400µL), PV2+CCl<sub>4</sub>: *Pistacia vera* (200 µL)+Carbon tetrachloride (100µL), PV4+CCl<sub>4</sub>: *Pistacia vera* (400 µL)+Carbon tetrachloride (100 µL). \*Each value is the mean±S.E. (standard error) of 5 repetitions. Superscripts after values in the same line with different letters represent significant differences. 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.

**Table 2: Fatty acid contents of herbal material in methanolic extracts (%).**

Fatty acids	<i>Pistacia vera</i>
C14:0 (Myristic Acid)	0.241
C14:1 (Myristic Acid)	0.101
C16:0 (Palmitic Acid)	9.360
C16:1n-7 (Palmitoleic Acid)	0.808
C18:0 (Stearic Acid)	2.373
C18:1 n-9 (Oleic Acid)	70.338
C18:2 n-6 (Linoleic Acid)	16.442
C18:3 n-6 (α - Linoleic Acid)	0.647
SFA	11.974
USFA	88.336
MUFA	71.146
PUFA	17.089

Σ: Total, ΣSFA: Total Saturated Fatty Acid, ΣUSFA: Total Unsaturated Fatty Acid, ΣMUFA: Total Monounsaturated Fatty Acid, ΣPUFA: Total Polyunsaturated Fatty Acid.

µL), PV2 (200 µL)+CCl<sub>4</sub> and PV4 (400 µL)+CCl<sub>4</sub> groups applied groups comparatively to control and CCl<sub>4</sub> groups (Figure 2).

The fatty acids are found to in the study, including 0.241% myristic (C14:0), 0.101% myristoleic (C14:1), 9.360% palmitic (C16:0), 0.808% palmitoleic (C16:1n-7), 2.373% stearic (C18:0), 70,338% oleic (C18:1n-9c), 16.442% linoleic (C18:2n-6c), and 0.647% linolenic (C18:3n-6) acids. There are variations in each of these fatty acids. With the analyses done on GC device fatty acids such as SFA, USFA, MUFA, PUFA were identified in the *Pistacia vera* fruit extract methanol extract (Table 2).

When the C16:1 n-7 (Palmitoleic acid) level was compared with control groups, statistically important variation was detected in PV2 and PV4 groups ( $p < 0.05$ ). When CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV<sub>4</sub>+CCl<sub>4</sub> group were compared with control group according to their fatty acid levels, it was found that the C16:1 n-7 level was statistically quite significant in CCl<sub>4</sub>

group, and there was a partially significant decrease in PV2+CCl<sub>4</sub> with PV4+CCl<sub>4</sub> groups ( $p < 0.001$ ;  $p < 0.01$ ) (Table 3).

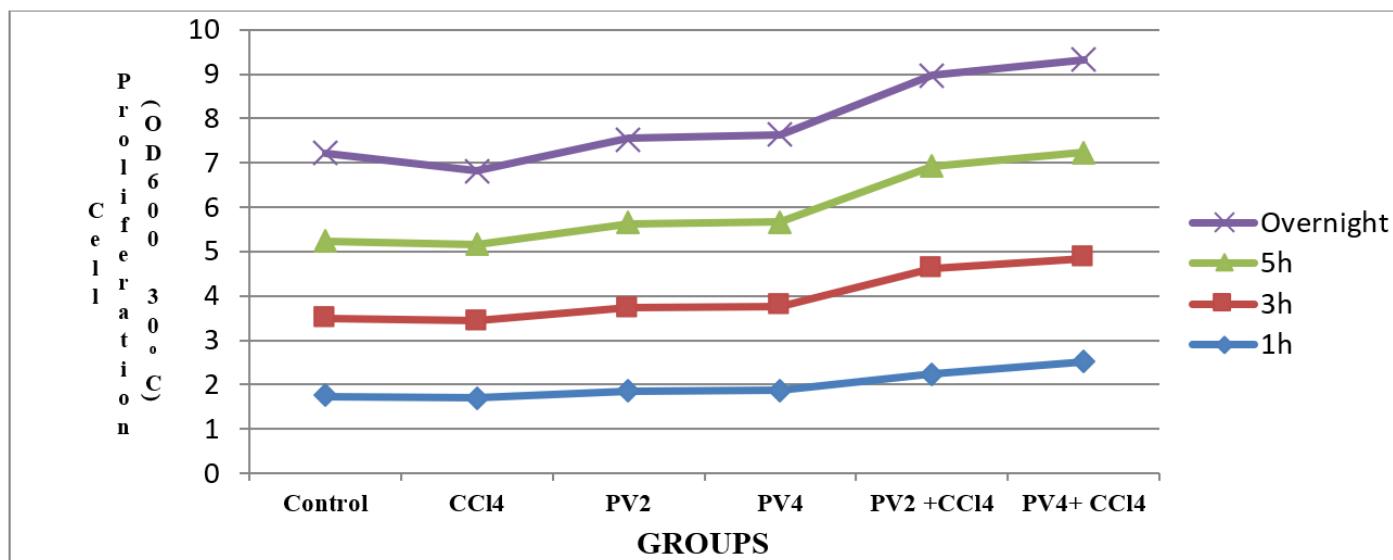
When CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> group were compared with control group according to their fatty acid levels, C18:1 n-9 (Oleic acid) level determined statistically important decrease in PV2+CCl<sub>4</sub> with PV4+CCl<sub>4</sub> groups and important decrease in CCl<sub>4</sub> group detected ( $p < 0.01$ ;  $p < 0.001$ ) (Table 3).

It was found that C18:2 n-6 (Linoleic acid) levels showed a statistically partial decrease in PV2, PV4 and PV4+CCl<sub>4</sub> groups contrary to the control group ( $p < 0.05$ ). The CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups, the C18:2 n-6 amount was statistically important decreased in CCl<sub>4</sub> group contrary to control group, while it showed a important decrease in PV4+CCl<sub>4</sub> group ( $p < 0.01$ ;  $p < 0.001$ ). C18:3 n-6 (α-Linoleic acid) level was not found to be statistically different in PV2, PV4 and PV4+CCl<sub>4</sub> groups contrary to control groups ( $p > 0.05$ ). When these controls with CCl<sub>4</sub> groups were compared, a statistically important decrease was observed at

**Table 3:** The effects of *Pistacia vera* and CCl<sub>4</sub> on the fatty acids amounts in the *S. cerevisiae* culture medium (mg/1 g)\*.

Fatty acids	Control	CCl <sub>4</sub>	PV2	PV4	PV2 +CCl <sub>4</sub>	PV4+ CCl <sub>4</sub>
C10:0 (Capric acid)	0.44±0.02 <sup>a</sup>	0.38±0.01 <sup>b</sup>	0.50±0.05 <sup>b</sup>	0.48±0.03 <sup>b</sup>	0.48±0.03 <sup>b</sup>	0.49±0.07 <sup>b</sup>
C12:0 (Lauric acid)	2.39±0.33 <sup>a</sup>	1.27±0.11 <sup>c</sup>	2.25±0.30 <sup>a</sup>	2.30±0.15 <sup>a</sup>	2.02±0.23 <sup>b</sup>	2.33±0.05 <sup>b</sup>
C14:0 (Myristic acid)	4.29±0.10 <sup>a</sup>	3.61±0.14 <sup>b</sup>	4.10±0.09 <sup>a</sup>	4.21±0.05 <sup>a</sup>	3.79±0.06 <sup>b</sup>	3.94±0.40 <sup>b</sup>
C16:0 (Palmitic acid)	44.76±0.51 <sup>a</sup>	25.36±0.51 <sup>d</sup>	40.56±0.48 <sup>b</sup>	42.88±0.51 <sup>b</sup>	37.24±0.30 <sup>c</sup>	38.96±0.46 <sup>c</sup>
C16:1n-7 (Palmitoleic acid)	8.9±0.23 <sup>a</sup>	5.24±0.11 <sup>d</sup>	7.10±0.09 <sup>b</sup>	7.83±0.12 <sup>b</sup>	6.06±0.11 <sup>c</sup>	6.41±0.19 <sup>c</sup>
C18:0 (Stearic acid)	6.87±0.08 <sup>a</sup>	4.12±0.06 <sup>c</sup>	5.71±0.06 <sup>b</sup>	6.23±0.01 <sup>a</sup>	5.21±0.04 <sup>c</sup>	5.97±0.07 <sup>b</sup>
C18:1 n-9 (Oleic acid)	51.87±0.79 <sup>a</sup>	27.60±0.31 <sup>d</sup>	41.14±0.87 <sup>c</sup>	48.28±0.59 <sup>b</sup>	36.64±0.22 <sup>c</sup>	40.48±0.94 <sup>c</sup>
C18:2 n-6 (Linoleic acid)	12.98±0.06 <sup>a</sup>	5.63±0.03 <sup>d</sup>	9.80±0.02 <sup>b</sup>	10.10±0.11 <sup>b</sup>	8.68±0.18 <sup>c</sup>	11.33±0.07 <sup>b</sup>
C18:3 n-6 (α-Linoleic acid)	14.82±0.05 <sup>a</sup>	6.51±0.02 <sup>d</sup>	13.59±0.01 <sup>a</sup>	14.65±0.06 <sup>a</sup>	12.54±0.08 <sup>b</sup>	13.95±0.07 <sup>a</sup>
Σ SFA	58.75±1.04 <sup>a</sup>	34.74±0.83 <sup>d</sup>	55.43±0.98 <sup>a</sup>	56.10±0.75 <sup>a</sup>	48.74±0.66 <sup>c</sup>	51.69±1.05 <sup>b</sup>
Σ MUFA	60.77±1.02 <sup>a</sup>	32.80±0.42 <sup>d</sup>	48.24±0.96 <sup>b</sup>	56.11±0.71 <sup>b</sup>	42.7±0.33 <sup>c</sup>	46.89±1.13 <sup>c</sup>
Σ PUFA	27.80±0.11 <sup>a</sup>	12.14±0.05 <sup>d</sup>	23.39±0.03 <sup>b</sup>	24.75±0.17 <sup>a</sup>	21.22±0.26 <sup>b</sup>	25.28±0.14 <sup>b</sup>
Total Fatty Acid	147.42±2.17 <sup>a</sup>	79.68±1.30 <sup>d</sup>	124.01±1.97 <sup>b</sup>	136.96±1.63 <sup>a</sup>	112.66±1.25 <sup>c</sup>	123.45±2.32 <sup>b</sup>

\* The meaning of the symbols is given under Table 1. Σ: Total, ΣSFA: Total Saturated Fatty Acid, ΣMUFA: Total Monounsaturated Fatty Acid, ΣPUFA: Total Polyunsaturated Fatty Acid.



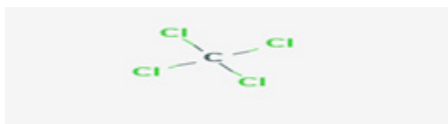
**Figure 1:** 1, 3, 5 hr and overnight (72 hr) *S. cerevisiae* cell growth density amounts for improving (OD<sub>600</sub> 30°C). CCl<sub>4</sub>: Carbon tetrachloride (100 µL), PV2: *Pistacia vera* (200 µL), PV4: *Pistacia vera* (400 µL), PV2+CCl<sub>4</sub>: *Pistacia vera* (200 µL)+Carbon tetrachloride (100 µL), PV4+CCl<sub>4</sub>: *Pistacia vera* (400 µL)+Carbon tetrachloride (100 µL).

the C18:3 n-6 level contrary to control groups ( $p < 0.001$ ) (Table 3).

When CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups were compared with control group according to their  $\Sigma$ SFA levels, statistically partial decrease in  $\Sigma$ SFA level was observed in PV4+CCl<sub>4</sub> group, an important decrease in PV2+CCl<sub>4</sub> groups and an important decrease in CCl<sub>4</sub> group ( $p < 0.05$ ;  $p < 0.01$ ;  $p < 0.01$ ). It was detected that  $\Sigma$ SFA level was not statistically different in PV2 with the PV4 group contrary to control ( $p > 0.05$ ). When  $\Sigma$ SFA with  $\Sigma$ MUFA level were compared to control groups, statistically partial decrease was determined in PV2 with PV4 group ( $p < 0.05$ ). When CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> group were contrary to control groups in terms of  $\Sigma$ SFA and  $\Sigma$ MUFA levels, it was found that there was a statistically important decrease in CCl<sub>4</sub> groups, PV2+CCl<sub>4</sub> with PV4+CCl<sub>4</sub> group ( $p < 0.001$ ;  $p < 0.01$ ). When CCl<sub>4</sub>, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups were contrary to control groups in terms of  $\Sigma$ PUFA levels, statistically partial decrease was determined in the PV2+CCl<sub>4</sub> with PV4+CCl<sub>4</sub> group ( $p < 0.05$ ). Total fatty acid level of the CCl<sub>4</sub> group showed a statistically important decrease in its level contrary to the control group ( $p < 0.001$ ) (Table 3).

## DISCUSSION

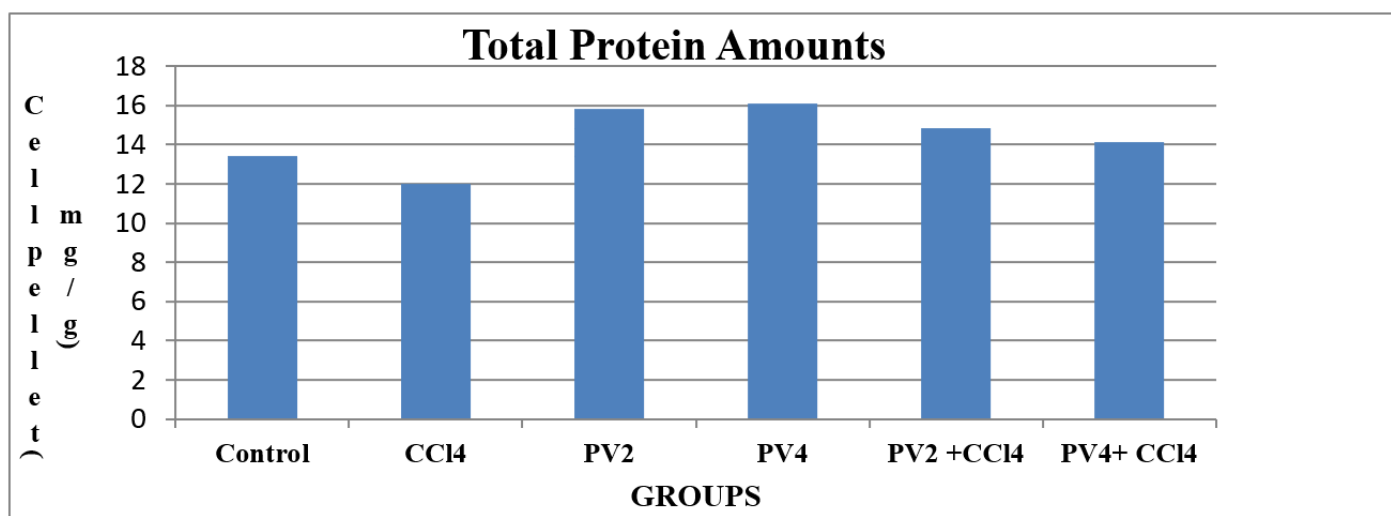
Important ROS in biological systems are produced by toxic contaminants. One of these, CCl<sub>4</sub>, is a dangerous material that is applied to various industry sectors.<sup>18</sup> Carbon Tetrachloride (CCl<sub>4</sub>) is frequently used from dry cleaning to pest control. It is an environmental toxin that results in reactive oxygen species in the biological system, which leads to oxidative stress.<sup>6,7</sup>



## Carbon Tetrachloride

According to clinical research, eating nuts can reduce your risk of developing cancer, diabetes, coronary heart disease, and low density lipoprotein.<sup>18,19</sup> Pistachio is also available in the literature that the protein content of pistachios, which is a potential protein source, is higher than hazelnut, walnut and pecan.<sup>20</sup> In parallel with these results, it is observed that the growth rate increased in the groups that added PV extract to the culture medium, especially when compared with CCl<sub>4</sub>. We can say that the reason for this increase is that the protein, vitamins, minerals, and antioxidants in the PV extract increase cell growth by reducing oxidative stress.<sup>6</sup> There was a statistically important variation between the groups, according to our analysis with various developmental stages. It was observed that Pistachio extract (PV) added to the culture medium increased cell growth against the negative effect of CCl<sub>4</sub> (Table 1, Figure 1). In this context, when the cell development results in Table 1 and Figure 1 and the total protein results given in Figure 2 is examined. We can say that PV extract stimulates protein synthesis in *S. cerevisiae*. A study by Moreno-Rojas *et al.* reported that pistachios are an important source of protein and dietary fiber.<sup>21</sup> This study again supports our findings.

Pistachio has a high fat content and is very rich in Unsaturated Fatty Acids (USFA). The quantity of Unsaturated Fatty Acid in total (USFA) is 87%, and Saturated (SFA) fatty acid ratio is 13%. Oleic acid (C18:1 n-9) is one of the most significant unsaturated fatty acids in pistachios. It constitutes more than 50% of the fatty acids in pistachios.<sup>22</sup> The ratio of fatty acids of the pistachio type used in the study and the pistachio type grown in the Kilis region we used in our study are similar (Table 2). According to the research conducted by Esteki *et al.*<sup>23</sup> the main components of pistachios were determined as saturated fatty acid palmitic acid (C16:0) and unsaturated fatty acids as oleic acid (C18:1)



**Figure 2:** The effects of *Pistacia vera* and CCl<sub>4</sub> on the total protein amount in the *S. cerevisiae* culture medium (mg/g) (cell pellet)\*. \* The meaning of the symbols is given under Figure 1.

and linoleic acid (C18:2). Oleic acid was predominant fatty acids, making up 56.88 with 57.75% of the raw with roasted pistachio oils, respectively linoleic acid made up 29.54 with 27.95% of the oil, respectively, and palmitic acid made up 10.23 and 10.93%.<sup>24</sup> Pistachios contain significant levels of  $\alpha$ -linoleic acid, one of the polyunsaturated fatty acids necessary for human health.<sup>25</sup> Pala *et al.*<sup>26</sup> found an average of 67.30% oleic acid, 17.83% linoleic acid, 9.65% palmitic acid, 2.94% stearic acid, and 2.04% palmitoleic acid in pistachio species examined. The fatty acid content of the pistachio type we used in our study showed parallelism with these studies (Table 2). In our study, it was determined that there were important modifications to the fatty acid profile quantities of yeast cells in which oxidative stress was induced by administering toxic substances (Table 3). In our findings, when the effects of PV extract on fatty acids were examined, it was determined that both the amounts and changes of fatty acids were at different values. In our study, reducing effects of CCl<sub>4</sub> application on many fatty acids were observed in *S. cerevisiae* (Table 3). It has been observed that various fatty acid metabolites accumulate or decrease in metabolic pathways for which enzymes are responsible, since the activity of enzymes is suppressed under disease conditions and when exposed to chemicals. Polyunsaturated fatty acid biosynthesis in mammals occurs via delta 5 desaturase and delta 6 desaturase from linoleic,  $\alpha$ -linolenic and oleic acids.<sup>27,28</sup> Compared to the CCl<sub>4</sub> group, some fatty acid levels of the PV2, PV4, PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> group, which were given PV extract, were increased compared to the groups that were treated with toxic substances (Table 3). This may be due to the fact that the chemical applied to the yeasts and the PV extract affect the metabolism of fatty acids. Because pistachio nuts are regarded as a highly significant dietary component and an excellent supply of unsaturated fatty acids, the most important of which is linoleic acid, of which 40-85% are MUFA and PUFA, the pistachio nut received significant attention.<sup>21</sup> In a study conducted by Granado-Casas *et al.* and Kalogeropoulos *et al.* found that pistachios are contain high levels of monounsaturated (73%) and polyunsaturated fatty acids (16%) including oleic acid (C18:1) (70%), which is known for its anti-inflammatory, hypocholesterolemic, and cardioprotective properties.<sup>29,30</sup> Nutritionists recommend pistachio eating as one of the elements of good dietary patterns. Mono- and polyunsaturated fatty acid, additionally unsaturated fatty acid with low levels of saturated fat, are abundant in these nuts.<sup>1,23,31</sup>

The results of this investigation showed that the palmitic (C16:0) level, which is an important fatty acid for metabolism, decreased significantly in CCl<sub>4</sub>-treated groups compared to control groups (Table 3). Within that case, we can say that it is caused by the effects of chemicals such as CCl<sub>4</sub> on acetyl CoA carboxylase and fatty acid synthetase activities, which are involved in lipid biosynthesis. Because at the end of the fatty acid production process is palmitic acid, it is synthesized by fatty acid synthetase and is released by some enzymatic reactions. Accordingly, the decrease in the amount of palmitic acid can be explained by the

decrease in fatty acid synthesis. According to the needs of the cells, palmitic acid is converted to palmitoleic acid (C16:1) by delta 9 desaturase (Stearoyl CoA) for the synthesis of phospholipid, sphingolipid, triglyceride, cholesterol or used in the synthesis of stearic acid (C18:0) by elongase. Stearoyl CoA Desaturase (SCD) is an enzyme that uses palmitic with stearic acids as substrate, and catalyzes formation of fatty acid with single double bonds for example, oleic acid from stearic acid and palmitoleic acid from palmitic acid.<sup>31</sup> The study, when the amount of palmitoleic acid was compared with control groups, it was observed that chemically administered CCl<sub>4</sub> groups decreased (Table 3). We think that this decrease is due to the inhibition of SCD activity. Again, in this research, it was observed that the amount of oleic acid (C18-1 n9) in CCl<sub>4</sub> groups decreased contrary to control groups (Table 3). This decline in quantity of oleic acid in yeast cell can be explained by decreased SCD activity, because SCD uses stearic acid as a substrate and converts it to oleic acid. In our study, the C18:2 n-6c (linoleic acid) levels was significantly lower at CCl<sub>4</sub> group than at control groups (Table 3). On the other hand, at CCl<sub>4</sub> applied groups, there was a decrease in  $\alpha$ -Linoleic acid level in these tissues at lower levels contrary to at control groups. We think that the activities of delta 6 and delta 5 desaturase enzymes, which are enzymes of the delta 6 desaturation metabolic pathway, decrease in tissues where the amount of  $\alpha$ -Linoleic acid decreases. Thus, it can be explained why the amount of  $\alpha$ -Linoleic acid changes in yeast cells.

## CONCLUSION

This study examined the fatty acid content of PV grown in the Kilis region in order to show preventive effects on a number of indices and against CCl<sub>4</sub>-induced cell damage in *S. cerevisiae*. When these findings are considered, PV has high fatty acid content. Nonetheless, the administration of PV extract exhibits a preventive effect by raising the enzyme activity against oxidative damage. It is believed to regulate the synthesis of fatty acids. In light of these results, we think that it has a special role in the production of fatty acids, which benefits yeast improvement. In light of our findings, we hypothesize that PV may have a similar effect on human health when considering how it affects yeasts. In addition, we believe that our research will advance scientific understanding of toxicity and natural products.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

CCl<sub>4</sub>: Carbon tetrachloride; ROS: Reactive oxygen species; BSA: Bovine Serum Albumin; SCD: Stearoyl CoA desaturase; GC: Gas Chromatography; PUFA: Polyunsaturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; USFA: Unsaturated Fatty Acid; SFA: Saturated Fatty Acid; Σ: Total; PV2: *Pistacia vera* (200 μL); PV4: *Pistacia vera* (400 μL); PV2+CCl<sub>4</sub>: *Pistacia vera* (200 μL)+Carbon tetrachloride (100 μL); PV4+CCl<sub>4</sub>: *Pistacia vera* (400 μL)+Carbon tetrachloride (100 μL).

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## SUMMARY

Pistachios are an important source of monounsaturated and polyunsaturated fatty acids, which have an important place in human nutrition. Therefore, their consumption has increased in recent years. Pistachios are rich in compounds that are beneficial for many metabolic diseases among nut species. This research, the fatty acid contents of *Pistacia vera* cultivated in Kilis province, the effects of this content on fatty acid profile and some biochemical parameters in CCl<sub>4</sub>-induced damage to cells in *Saccharomyces cerevisiae* (bread yeast) were investigated. A cell culture model based on *S. cerevisiae* (bread yeast) was used. There were six groups in this study. i) *Pistacia vera* 200 μL (PV2) groups; ii) Carbon Tetrachloride 100 μL (CCl<sub>4</sub>) groups, iii) *Pistacia vera* 200 μL (PV2) groups, iv) *Pistacia vera* 400 μL (PV4) groups; v) PV2+CCl<sub>4</sub> groups; and vi) PV4+CCl<sub>4</sub> groups. The results showed that, in contrast to the CCl<sub>4</sub> group, total protein synthesis and cell proliferation increased in PV2+CCl<sub>4</sub> and PV4+CCl<sub>4</sub> groups at one, 1,3,5 and 72 hr (overnight). Palmitic (C16:0) level, which is an important fatty acid for metabolism, was importantly decreased in CCl<sub>4</sub> treated groups contrary to control groups ( $p < 0.001$ ). This research, it was observed that the quantities of palmitoleic acid (C16:1) with oleic acid (C18-1 n9) decreased the CCl<sub>4</sub> treated groups in contrast to the group under control ( $p < 0.001$ ;  $p < 0.01$ ). In summary, PV extract helps *S. cerevisiae* culture by lowering oxidative damage caused by CCl<sub>4</sub> and promoting cell growth and total protein synthesis. The positive results of PV extract especially on fatty acid biosynthesis and responsible enzyme activities will provide a basis for related research on different live models. The results of this study may be supported in more detail by future clinical investigations.

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