Progress in Nutrition 2020; Vol. 22, N. 4: e2020077 DOI: 10.23751/pn.v22i4.9901

Original article

The protective effect of *pistacia vera* L. (Pistachio) against to carbon tetrachloride (CCl_4)-induced damage in *saccharomyces cerevisiae*

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Summary. In this study, phytochemical ingredients of *Pistacia vera* cultivated in Kilis province, and it was investigated to demonstrate anti-oxidant effects and on some biochemical parameters and of this content on CCl_4-induced cell damage in *Saccharomyces cerevisiae. Pistacia vera* fruits were extracted and then subjected to flavonoid analysis by HPLC device. *S. cerevisiae* (bread yeast) was used as a cell culture model. For the development and proliferation of *S. cerevisiae* in the study, YEDP (1 g yeast extract for 100 ml, 2 g bacto-peptone, 2 g glucose) medium was used. In this study, six groups were composed. i: Control group, ii: CCl4 (100 µl) group, iii: *Pistacia vera* 200 µl (PV2) group, iv: *Pistacia vera* 400 µl (PV4) group, v: PV2+CCl4 group and vi: PV4+CCl4 group. After sterilization, PV and CCl4 were inserted to *S. cerevisiae* cultures and the cultures were developed at 30°C for 72 hour. Antioxidant enzymes activities such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSHpx), glutathione S-transferase (GST) and glutathione reductase (GR) were determined in *S. cerevisiae* cell by spectrophotometer. Glutathione (GSH), oxidized glutathione (GSSG) and malondialdehyde (MDA) were investigated in *S. cerevisiae* cell by HPLC device. In this study, it is determined that pistachio is rich in flavonoid. Other results indicated that CCl4 application significantly increased MDA and GSSG contents the most significantly ($p<0.001$). However, the GSH level in comparison to control group decreased the more significantly in CCl4 group ($p<0.001$). In the groups given PV extract against CCl4, it was found that the GSH levels increased and MDA level reduced in *S. cerevisiae cell* ($p<0.05$, $p<0.01$). According to the results in comparison to control group, the activity of SOD, GST, GSH-Px, CAT and GR were decreased significantly with CCl4 treatment ($p<0.01$). In our data in comparison to CCl4 group, the activity of SOD, GST, GSH-Px, CAT and GR were increased significantly in PV2+CCl4 and PV4+CCl4 groups with treatment PV extract ($p<0.05$, $p<0.01$). Our study shows that PV is very rich in flavonoids. As a result, PV extract has a role in promoting cell growth as well as reducing oxidative damage in *S. cerevisiae culture*. In particular, the results of the antioxidant defense system will be a source for similar studies on other living models.

Key words: *Saccharomyces cerevisiae,* Pistacia vera, CCl4, Oxidative damage, Antioxidant systems, Flavonoid.

Introduction

The genus Pistachio which involves 11 species (e.g. *Pistacia vera*L., *P. terebinthus*L., *P. lentiscus* L., etc.), pertains to the Anacardiaceae family (1). Between these species, *Pistacia vera* (PV) is solely an economic and cultivated kind (2).

Pistacia vera L. (pistachio) is one of the most delicious and nutritious nuts a good source of useful health nutrients like vitamins, antioxidants, proteins, and phenols (3,4). This nut is originally from Middle East, and there are four main producers of pistachios including United States, Iran, Turkey, and China (5). Of all these nations, Turkey is the third biggest importer and exporter of pistachio nuts (6). Pistachio is generally consumed as a snack. In addition, it is used to increase color and taste in desserts such as cake, chocolate, baklava, ice cream and meat products such as sausage, salami and banger (7,8).

PV, which is a rich source in terms of phenolic compounds, draws attention with its high antioxidant activity. PV is a rich source of oil soluble antioxidants which have a significant effect upon the control of oxidative stress and reducing the risk of chronic diseases (9, 10). Oxidative stress and production of free radicals increase by various factors such as toxic effects of xenobiotics or ionized radiation. (11). Carbon tetrachloride (CCl_4) is used widely as intermediate product in the production of cleansers and solvents, disinfection of cereals and in the synthesis of chlorofluorocarbons. It is an environmental toxin that causes oxidative stress by producing reactive oxygen species in the living system. Toxic effect of CCl_4 is closely related to metabolic activation of short-life reagent intermediate products (12). Fragmentization products (mostly reactive aldehydes) cause further damage by accumulating in the cell. The damaging effect of CCl_4 is associated with the development of oxidative stress, where in severe cases can lead to destruction of intracellular mitochondrial and lysosome membranes, protein denaturation and cell death (13).

Saccharomyces cerevisiae cell will be used in our study for these reasons because *S. cerevisiae* takes place in the most important cell model (14-16).This cell module is generally used in xenobiotic, toxicological, biochemical and molecular studies. Therefore, yeasts show resistance against toxic substances thanks to their various properties. This resistance may show its effect with different mechanisms in the form of metal intake or reduction of its passage or cell retention of metals. Although there are some differences in metabolic characteristics that these yeast cells have, it has been determined that many of their properties seem similarity to advanced organisms and parallelism in metabolic reactions and given xenobiotics (17). However, it was not found any study about biochemical effects of PV extract on *S. cerevisiae* cells exposed to CCl_4 among the studies conducted till today.

In this study, phytochemical ingredients of PV samples cultivated in Kilis province the effects of CCl_4 and PV extract added to the development environment of *S. cerevisiae* on some biochemical parameters and in the antioxidant defense system were investigated.

Material and Methods

Herbal materials

The Fruit of PV was used as herbal materials and they were obtained from Musabeyli in Kilis, Turkey. The fruit of PV was extracted with 85% methanol.

Flavonoid analysis of fruit extract

In the methanolic extracts of the flavonoids of fruit were analysed by PREVAIL C18 (15 x 4.6 mm, 5 μm) HPLC column. Methanol/water/acetonitrile mix (46/46/8, v/v/v) containing 1% acetic acid was used as the mobile phase (18). Results were expressed as μg/g.

Research groups and growth conditions

Six groups of media in 5 parallels were composed; i: Control group, ii: CCl_4 (100 μl) group, iii: *Pistacia vera*200 μl (PV2) group, iv: *Pistacia vera* 400 μl (PV4) group, v: PV2+CCl_4 group and vi: PV4+CCl_4 group. After sterilization, PV and CCl_4 were inserted to *S. cerevisiae* cultures and the cultures were developed at 30°C 72 hours (19). At the end of incubation, the samples were centrifuged in a cooling centrifuge (5000 rpm +4 °C'de 5 min). The pellets were obtained by

centrifuge and were weighed. The pellets were washed by the 50 mM KH_2PO_4 solution. The pellets were homogenized in cold medium with 10 mL of 50 mM Tris-20 mM EDTA (pH = 7.40) buffer mixture. After this procedure, they were centrifuged in a cooling centrifuge (9000 rpm +4 ºC'de 5 min). The supernatants were obtained and separated for MDA, GSH, GSSG and antioxidant enzyme analyzes.

Measurement of lipid peroxidation (MDA) level

The measurement of MDA levels were analyzed on the HPLC device (20). Supernatant 1 mL was taken and 10% perchloric acid (PCA) added. After centrifuged in a cooling centrifuge (5000 rpm +4 ºC'de 5 min) the supernatant part was taken into autosampler vials and analyzed in HPLC. Shimadzu full VP series HPLC device was used for analysis. According to the method of Karatepe et al., (2004) was used mixture of 30 mmol KH_2PO_4 and methyl alcohol as mobile phase (82.5-17.5%, pH = 4.0 with H_3PO_4) ODS-3 HPLC column as the colon (150 mm x 4.6 - 5 µm) (21)

GSH and GSSG amount measurement

The measurement of GSH and GSSG amounts were analyzed on the HPLC device (22,23). Supernatant 1 mL was taken and 10% trichloroacetic acid (TCA) added. After centrifuged in a cooling centrifuge (5000 rpm +4 ºC'de 5 min) the supernatant part was taken into autosampler vials and analyzed in HPLC.Pump LC–10 AD^{VP}UV-visible, detector SPD-M10A^{VP}PDA, column furnace CTO-10AS^{VP}, autosampler SIL-10AD^{VP}, degasser unit DGU-14A and Class VP 6.26 operating program (Shimadzu, Kyota Japan) were used in the device. A mixture of 0.1% trifluoroacetic acid (TFA) and methanol (94%/6%, v/v) was used as mobile phase. The separation was made with the ODS-3 HPLC column.

Antioxidant enzymes analysis

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed in terms of its ability to inhibit the oxygen-dependent oxidation of adrenalin (epinephrine) to adenochrome by xanthine oxidase plus xanthine (24). The reaction was followed at 480 nm and one unit of SOD activity is defined as the amount of the enzyme causing 50% inhibition of the rate of adenochrome production at 26°C. Solutions used in SOD activity measurement was made fresh daily. The assays were run by adding to the cuvette sequentially 0.05 M potassium phosphate buffer pH 7.8/0.1 mM EDTA, 100 µl adrenaline, 100 µl xanthine and 200 µl sample. The reaction was then initiated by adding 20 µl xanthine oxidase.

Catalase (CAT) (EC 1.11.1.6) activity was measured by following the reduction of hydrogen peroxide (H_2O_2) at 30°C and 240 nm using the extinction coefficient 0.04 mM-1 cm-1 (25). Immediately before assay, a stock solution was prepared. The quartz assay cuvette contained 50µl sample solution in a final volume of 250 µl containing 67 mM phosphate buffer pH 7.0 and 20 mM H_2O_2. One unit of CAT represents the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute.

Glutathioneperoxidase (GSHpx) (EC 1.11.1.9) was assayed by following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase (26). The GSSG generated by GSHpx was reduced by GR and NADPH oxidation was monitored at 340 nm. The quartz assay cuvette contained the reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.1), 1 mM EDTA, 3.6 mM reduced glutathione (GSH), 3.6 mM sodium azide, 1 IU ml–1 glutathione reductase, 0.2 mM NADPH and 0.05 mM H2O2. Moreover, 0.05 mM cumene hydroperoxide was used as substrate instead of hydrogen peroxide. Sample was added and specific activities were determined using the extinction coefficient of 6.22 mM-1 cm-1.

Glutathione reductase (GR) (EC 1.6.4.2) activity was determined by the oxidation of NADPH at 340 nm using the extinction coefficient 6.22 mM-1 cm-1. Reaction mixture in quartz assay cuvette consisted of 0.1 M potassium phosphate buffer (pH 7.2), 2 mM EDTA, 0.63 mM NADPH and 0.15 mM oxidised glutathione (GSSG). The reaction initiated by the addition of the sample (27).

Glutathione S-transferase (GST) (EC 2,5,1,18) activity was measured at 340 nm with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM

glutathione (GSH) in 100 mM potassium phosphate buffer, pH 6.5. The quartz assay cuvette containing 100 mM potassium phosphate buffer pH 6.5. 100 ml GSH and 100 ml CDNB were prepared and the reaction initiated by the addition of 50 ml sample. Specific activities were determined using an extinction coefficient of 9.6 mM-1 cm-1 (28).

Data analysis

SPSS 15.0 Software was used to data analysis. Comparison between control group with experimental groups was made the analyzes of variance (ANOVA) with LSD tests. The results were presented as mean ± SEM. For determining the difference between the groups, $p > 0.05$, $p < 0.05$, $p < 0.01$, with $p < 0.001$ values were used.

Results

Flavonoids analyses that were performed using the HPLC device showed the methanol extract of PV (Table 1). According to the results of flavonoid analysis catechin, epigallocatechin, luteolin, and rutin flavonoids were high in PV sample, whereas other flavonoids were present at lower levels (Table 1).

The MDA level in the cell pellets in the *S. cerevisiae* culture medium increased in all groups given CCl_4 in comparison to the control group. This increase in comparison to the control group was highly significant in the CCl_4 group ($p<0.01$). The increase in the MDA levels in the PV2+CCl_4 and PV4+CCl_4 groups was significant in comparison to the control group ($p<0.05$) (Table 2). The GSH level in the cell pellets in the *S. cerevisiae* culture medium decreased in CCl_4 group in comparison to the control group ($p<0.001$). In terms of the GSH levels, the increase in comparison to the control group was noticeable in the PV2 and PV4 groups and highly significant PV2+CCl_4 and PV4+CCl_4 in the groups ($p<0.01$) ($p<0.05$) (Table 2). The GSSG level in the cell pellets in the *S. cerevisiae* culture medium increased in all groups given CCl_4 in comparison to the control group. This increase in comparison to the control group was highly significant in the CCl_4 group ($p<0.001$). The increase in the GSSG levels in the PV2+CCl_4 and PV4+CCl_4 groups in comparison to the control group was significant ($p<0.01$) (Table 2).

When the antioxidant enzyme activities of the cell pellets in the *S. cerevisiae* culture medium were examined, the enzyme amounts were observed to decrease in all groups given CCl_4 (Table 3). The amount of SOD increased in comparison to the CCl_4 group significantly PV2+CCl_4 group and in the PV4+CCl_4 group ($p<0.01$). There was significant difference in terms of the SOD levels in the PV2 and PV4 groups in comparison to the control group ($p<0.05$) (Table 3). The GST levels increased in comparison to the CCl_4 group significantly difference in the PV2+CCl_4 and PV4+CCl_4 groups ($p<0.05$). There was a significant increase in the GST levels in the PV2 and PV4 groups in comparison to the control group ($p<0.05$) (Table 3). The CAT amounts in the PV2+CCl_4 and PV4+CCl_4 groups showed a significant increase in comparison to the control group ($p<0.05$). Moreover, there was a significant increase in the CAT amounts in the PV2 and PV4 groups in comparison to the control group ($p<0.05$) (Table 3). In comparison to the control group, the GR levels increased noticeably in the CCl_4 group and highly significantly in the PV2+CCl_4 and PV4+CCl_4 groups ($p<0.01$) ($p<0.001$) (Table 3). The GR levels in the PV2 and PV4 groups showed an insignificant increase in comparison to the control group ($p>0.05$) (Table 3). The GSHpx amounts in the CCl_4 groups showed a highly significant decrease in comparison to the control group ($p<0.001$). Moreover, there was a significant increase in the GSHpx amounts in the PV2 and PV4 groups in comparison to the control group ($p<0.05$) (Table 3).

Table 1. Flavonoid contents of *Pistacia vera* in methanolic extracts (μg/g)

Flavonoids	***Pistacia vera***
Luteolin	3.81
Cathechin	5.02
Rutin	2.17
Myricetin	0.46
Morin	0.25
Naringenin	0.23
Quercetin	1.51
Epicatechin	1.02
Epigallocatechin	3.04

Table 2. The effects of *Pistacia vera* and CCl_4 on the MDA, GSH and GSSG amounts in the *S. cerevisiae* culture medium (cell pellet)*

GROUPS	**MDA** (nmol/g)	**GSH** (µg/g)	**GSSG** (µg/g)
Control	30,73±0,21[a]	138,69±2,39[a]	18,69±0,72[a]
CCl_4	44,29±0,19[d]	95,49±0,46[d]	35,63±0,73[d]
PV2	23,41±0,40[b]	142,39±0,61[c]	21,20±0,40[b]
PV4	24,56±0,60[b]	145,48±1,57[c]	23,12±0,64[b]
PV2 +CCl_4	33,84±0,37[b]	140,53±2,30[b]	33,90±0,38[c]
PV4+ CCl_4	32,04±0,36[b]	141,62±2,63[b]	34,82±0,35[c]

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).

*Each value is the mean±S.E. (standard error) of 5 repetitions. Superscripts after values in a same line with different letters represent 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

MDA: Malondialdehyde **GSH:** Glutathione **GSSG:** Oxidizedglutathione

Table 3. Effects of application groups on antioxidant enzyme activity examined in *S. cerevisiae* (cell pellet)*

GROUPS	**SOD** (U/g)	**GST** (µg/g/1min)	**CAT** (µg/g/1min)	**GR** (U/g/1min)	**GSHpx** (U/g/1min)
Control	18,53±0,32[a]	1,75±0,21[a]	13,60±0,13[a]	0,24±0,17[a]	10,46±0,14[a]
CCl_4	13,18±0,30[c]	0,97±0,23[c]	8,63±0,26[c]	0,10±0,08[c]	8,34±0,12[c]
PV2	17,47±0,39[b]	1,35±0,11[b]	10,38±0,16[b]	0,18±0,03[b]	12,43±0,15[b]
PV4	17,50±0,34[b]	1,40±0,19[b]	11,71±0,05[b]	0,19±0,05[b]	12,14±0,23[b]
PV2 +CCl_4	16,02±0,60[b]	1,30±0,10[b]	10,23±0,11[b]	0,15±0,18[c]	10,71±0,27[b]
PV4+ CCl_4	15,98±0,27[b]	1,34±0,06[b]	10,05±0,30[b]	0,17±0,19[c]	11,83±0,1[1b]

* The meaning of the symbols is given under Table 2.

SOD: Superoxidedismutase **GST:**GlutathioneS-transferase **CAT:**Catalase **GR:**Glutathionereductase **GSHpx:** Glutathioneperoxidase

Discussion

Due to the fact that nuts contain tocopherol, omega-3 compounds, polyphenolic and phytochemicals, the interest in their consumption is increasing day by day (29). Of these, *Pistacia vera* nut is one of the most frequently used tree nuts in the world due to its special organoleptic characteristics (30). Clinical investigation have shown a positive correlation between intake of nuts and prevention of diabetes, coronary heart diseases, low density lipoprotein and cancer (31).

Toxic pollutants are the sources of important ROS in biological systems. Of these, CCl_4 is a toxic substance used in different branches of the industry. It is an environmental toxin that causes oxidative stress by producing reactive oxygen species in the living system (12).

S. cerevisiae is anaerobic under normal conditions, but can survive or even reproduce under aerobic conditions. Aerobic organisms have both chemical and enzymatic defense systems against ROS-induced toxicity (32,33). Lifelong, people are exposed to different chemicals originating from various sources (34). In the literature search, *S. cerevisiae* was used to determine the mechanism of action of chemicals such as heavy metals and hydrogen peroxide (35,36). However, no study has been found on *S. cerevisiae* regarding the effect of CCl_4, which is a highly reactive toxic substance for living systems.

When our body is exposed to CCl_4, it is metabolized by cytochrome P450 2E1 and leads to formation of free radicals, oxidative stress and inflammatory processes. Current studies reported that CCl_4 leads to oxidative stress and lipid peroxidation formation, and as a result of this, it plays a significant role in the formation of diffuse cellular and tissue damage by cell damage and cellular enzymes to transfer into the blood (37,38). MDA is the end product of lipid peroxidation and an indicator of cellular damage (39). In our study, it was seen that the MDA levels of yeast cells exposed to CCl_4 application increased in comparison to the control group (Table 2). According to studies that are conducted, the increase in MDA levels by substances that lead to free radical formation in living beings such as heavy metals and hydrogen peroxide was in parallel to the increase in our study (40, 41). Additionally, in our study, a decrease was determined in the MDA levels in the groups given PV extract in comparison to the CCl_4 groups. However, this decrease was limited despite the provision of high concentrations of PV extract. According to our results, PV extract could protect the *S. cerevisiae* cell against the cell damage caused by oxidative stress. This protective activity of PV is probably caused by its contents including flavonoids which have antioxidant properties (Tables 1). In CCl_4 studies with different experimental models and organisms, it was determined that MDA levels increased, and this increase was reduced by application of various plant extracts (42,43). Moreover, Aydın et al. (2015) applied an extract of *Juglans regia* L. (walnut) to male Wistar rats against CCl_4, and a significant reduction in the MDA levels was observed as a response effects to oxidative stress. Likewise, the authors applied the same extract on *S. cerevisiae* and determined that the MDA levels also decreased significantly here (44). This information in the literature is in parallel to the results of our study (Table 2). As can be seen, the positive effects of plant extracts have been observed in scientific researches carried out on yeasts as well as rats.

There are several studies on the enzyme activities of extracts that are obtained from various parts of plants by using various organic solvents. Enzymes are highly important in biological systems, and they play an important role in the treatment and diagnosis of several diseases (45).

GSH is an important non-enzymatic antioxidant. With this antioxidant property, GSH protects cells from oxidative damage through inactivating xenobiotics by entering into a reaction with free radicals and peroxides (46). In this study, it was determined that the GSH level decreased in the CCl_4 group, while the GSH levels in the groups where pistachio extract was applied increased significantly in comparison to the control group. This increase that was observed in the GSH levels was the highest in the PV2C group (Table 2). The reason for this increase can be considered as the development of a defense mechanism against the toxicity of CCl_4 by *S. cerevisiae's* PV extract application. It can be explained by the adaptation of S. cerevisiae in this case against oxidative damage. This view of ours was supported by the study conducted by Gokce (2013**)** which reported similar responses to oxidative stress after trying CCl_4 that causes oxidative stress and different extracts on yeasts and experimental animals (11). Moreover, Aydın et al. (2015) applied an extract of *Juglans regia* L. (walnut) to male Wistar rats against CCl_4, and glutathione synthesis increased as a response to oxidative stress (44). Likewise, the authors applied the same extract on *S. cerevisiae* and determined that the GSH levels also increased significantly here (47). This information in the literature was in parallel to the results of our study (Table 2).

In this study, it was determined that the oxidized glutathione (GSSG) content increased, and the GSH levels decreased in the CCl_4 group in comparison to the control group (Table 2). Increases in parallel to the reductions in the glutathione content were observed in the oxidized glutathione content. As a result of the adaptation mechanisms that are activated in the case that oxidative stress is weak, the GSH level is increased. However, in the case that oxidative stress is strong, the GSH level decreases based on weakened adaptation mechanisms and increased GSSG formation (48). The enzyme in the cell that is responsible for catalyzing the reduction of GSSG into GSH is known as glutathione disulfide reductase (G; EC 1.6.4.2). The situation of an increase in the GSSG amounts indicates a disorder in the NADPH production pathways or means a decrease in the intracellular GSH amounts as a result of the inactivation of the enzyme. This view of ours was supported by the study conducted by Izawa et al. (1995) (49). These researchers reported that one of the adaptation mechanisms formed against H_2O_2 may be intracellular glutathione increase. As a result of our study, it was determined that the glutathione amount decreased, and the GSSG amount increased in the CCl_4 group in comparison to the control group (Table 3).When this result is considered together with antioxidant enzymes and MDA contents, it supported the idea that CCl_4 induces severe oxidative stress in the environment as in the case of other chemicals, and this was in agreement with the literature (12). Additionally, Aydın (2012) applied an extract of *Juglans regia* L. (walnut) against CCl_4 in *S. cerevisiae* and determined that the GSH level significantly increased as a response to oxidative stress our results were in parallel with their findings (47). Moreover, the GSSG levels increased in the CCl_4 groups given different concentrations of PV extract. However, increasing the concentration of the extract did not affect the increase in GSSG. This showed us that the extract has a limited protective effect against oxidative stress. This protective effects of PV may be explained by the flavonoid and vitamin derivatives in its contents (Table 1).

Oxidative stress cause damage in cell and tissue. Antioxidant enzymes SOD, CAT, GR and GST are major scavenging enzymes that represent protection against oxidative stress. SOD catalyzes the dismutation of superoxide anion to a hydrogen peroxide. GR enzyme intracellular glutathione regulates metabolism. CAT, GSH-Px H_2O_2 to non-toxic products H_2O and O_2 GST neutralizes cellular peroxides. Xenobiotics are oxygenated with this enzyme system (42**)**.

Table 3 shows the change in level of SOD, CAT, GR, GST and GSH-Px after different treatments. In comparison to control group, the enzymes activity was decreased in the CCl4 group of in *S. cerevisiae*. The protective effects of PV extract on SOD, CAT, GR, GST and GSH-Px in *S. cerevisiae* are shown in Table 3. Treatment with PV extract recovered the normal level of these enzymes. These results show that CCl4 causes oxidative stress in the yeast cell. It shows protective effect by increasing the enzyme activity against oxidative damage with the application of PV extract. We think that this protective effect is due to the phytochemical content of the plant (Table 1). Pistachio as known present high amounts of phenolic compounds which can exert beneficial health effects against different diseases (10). In addition Lim (2012) have emphasize consumption of pistachio nuts decreased oxidative stress (50).

Research on experimental animals is consistent with our study. However, no study on yeast cell has been found. For example (10) the effects of ground pistachio kernel extracts have been evaluated on cellular viability, ROS production and cell death in MCF-7 breast cancer cells. In the study, Pistachio kernel extract showed promising beneficial effects that could be exploited as a "natural adjuvant" in combination with chemotherapy treatment. Another example is protective effect of ethanol extract of *Pistacia chinensis* was investigated in rats against CCl_4 induced lung and thyroid injuries. The levels of antioxidant enzymes, that is, catalase, peroxidase, superoxide dismutase, glutathione-S-transferase, glutathione reductase, and glutathione peroxidase in both lung and thyroid tissues of CCl_4 treated rats. In conclusion, *P. chinensis* exhibited antioxidant activity by the presence of free radical quenching constituents (51).

Conclusion

In this research, phytochemical ingredients of PV cultivated in Kilis province, and it was investigated to demonstrate anti-oxidant effects and on some parameters and of this content on CCl_4-induced cell damage in *S.cerevisiae*. When these results are evaluated; PV is very rich in flavonoids. However it shows protective effect by increasing the enzyme activity against oxidative damage with the application of PV extract. With respect to these findings, we believe that it has particularly reduced oxidative damage in this way making a positive influence on yeast improving, with respect to our results, we suppose that PV possibly a like impact on human health when we imagine its effect mechanism on yeasts. At the same time, we think that our study will contribute to scientific knowledge in terms of natural products and toxicology.

Acknowledgments

This work was supported by the Kilis 7 Aralık University Project Support and Coordination (BAP Project number 12352).

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