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Original article

# Influences of *Pistacia terebinthus* L. and *Rhus coriaria* L. fruits extracts on the levels of some biochemical parameters in liver tissue of Experimental Breast Cancer rats

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**Summary.** In this study, against to DMBA-induced breast cancer healing effects of *Pistacia terebinthus* L. subsp. *Palaestina* (terebinth) and *Rhus coriaria* L. (sumac) were examined as biochemical in the female Sprague-Dawley rats. 66 rats were used in this study. Rats were divided into 6 groups as Control, DMBA, PT, RC, PT+DMBA and RC+DMBA. DMBA was administered by gavaged, a single dose of 80 mg/kg according to body weight, to 8-week-old rats. Aqueous extracts of terebinth and sumac were given orally 3 days a week to rats in antioxidant groups. Malondialdehyde, glutathione, vitamin, cholesterol, antioxidant enzyme and fatty acid levels, which are signs of lipid peroxidation, were measured in liver tissue. At the end of the study in cancerous rats, malondialdehyde increased compared to the control group. Glutathione decreased in the other tissues and all of the DMBA-induced cancerous rats groups. Lipophilic vitamins and cholesterol levels were analyzed by HPLC. The levels were different in DMBA and antioxidant groups of tissues. In cancerous rats, liver cholesterol levels decreased. The results of the present study showed that the herb suspensions exerted anti-cancer effects and consequently may alleviative liver damage caused by DMBA-induced breast cancer.

**Key Words:** Breast cancer, *Rhus coriaria*, *Pistacia terebinthus*, Liver tissue, Lipid peroxidation, Antioxidant enzyme, Fatty acid

## Introduction

Breast cancer is the most common cancer that causes death in women worldwide. GLOBOCAN (1) has been stated that breast cancer mortality in women worldwide is 11.6%.Breast cancer treatment includes surgery, radiotherapy, chemotherapy and immunotherapy. New chemotherapeutic agents and molecular targeted drugs contribut to cancer treatment. However, the toxicity and drug resistance of these substances also can result in the failure of chemotherapy. For this reason, researchers are trying to discover less toxic and effective biocomponents for treatment. To this end, medicinal plants have become the focus for the development of new anticancer agents (2).

Numerous traditional herbal remedies are used by cancer patients, especially in the third World countries and may, therefore, represent new avenues in the search for alternative carcinogenic drugs (3).

Plant-derived anti-cancer drugs are more effective than synthetic drugs and have fewer side effects. Examples of anti-cancer drugs derived from plants is

the vinblastin and vincristine contain vinca alkaloids, currently used in the clinic, isolated from Catharan roseus, the paclitaxel terpenes from Taxusbrevifolia and the camptothecin that an inhibitor of DNA topoisomerase I, from *Camptotheca acuminata* (4).

In recent times, *Pistachia terebinthus* L. and *Rhus coriaria* L.are very much appreciated by researchers worldwide for their effect as anti-cancer agents. Medically, *Pistachia terebinthus*. and *Rhus coriaria* have been used as a medicinal herb to cancer, leukemia, asthma, hepatitis or diabetes (2,5,6). Besides, there is no information about if exposure to *P. terebinthus* and *R. coriaria* extracts of biochemical parameters in liver tissue of cancer rats.

The present study was conducted to determine biochemical activity of *P. terebinthus* and *R. coriaria* extracts in liver tissue of breast cancer rats.

## Materials and Methods

### *Animals*

Animals, experimental design, and experimental protocols were approved by the local Animal Experiments Ethics Committees of Firat University (Elazig, Turkey). Animal care and experimental protocols were compatible with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 12.10.2016/181). Sixty-six healthy adult female Sprague-Dawley rats, aged 8 weeks were obtained from Firat University Experimental Research Centre (Elazig, Turkey). The animals were housed in the polycarbonate cages in a room with a 12-h day-night cycle, temperature of 22±3°C, and humidity of 45% to 65%. During the whole experimental period, the animals were fed with a balanced commercial diet (Elazig Food Company, Elazig, Turkey) *ad libitum*.

### *Experimental design*

The first group was used as the control group (n=7) and the others were as follows: second group, DMBA (n=15); third group, *Pistacia terebinthus* group (PT) (n=7); fourth group, *Rhus coriaria* (RC) (n=7); fifth group, DMBA+ *Pistacia terebinthus* group (DMBA+PT) (n=15); sixth group, DMBA+ *Rhus coriaria* group (DMBA+RC) (n=15) groups were made carcinogen by single dose with gavage of 80 mg/kg 7,12-dimethylbenz(α)anthracene (DMBA) (7).

The rats in plant groups were added of 20 mg/kg *Pistacia terebinthus* and *Rhus coriaria* extracts to 500 ml drinking water one times per week. These treatments continued for 16 weeks and after this period, each experimental rat was anesthetized with ether. Liver tissue samples were dissected and stored at -85°C prior to biochemical analyses.

### *Homogenate preparation*

Tissue samples were homogenized in Tris-HCl buffer (pH 7.4) and centrifuged at 9050xgfor 15 min at 4°C. Supernatants were collected, aliquoted, and stored at -70°C until use. The supernatant obtained from the MDA, glutathione (GSH), antioxidant enzymes (CAT, SOD and GST) and total protein analysis, the pellets ADEK vitamins, cholesterol, and fatty acid analysis was performed.

### *Determination of MDA-TBA level*

Lipid peroxides (TBARS) in tissues homogenate were estimated using thiobarbituric acid reactive substances by the method of Ohkawa et al. (8). To 1,0 ml tissue homogenate, 0,5 ml of 8,1% SDS, 1,0 ml of (20% acetic acid/NaOH pH 3,5), 1,0 ml of 10% TCA, 50 µl of 2% BHT and 1,0 ml of 0,8% TBA were added. The mixture was heated in a water bath at 95°C for 60 min. After cooling, 4 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4250 rpm for 15 min, the organic layer was taken and its absorbance at 532 nm was measured. 1.1.3.3-tetramethoxypropane was used as standard. The resulting nmol/g tissue was calculated.

### *Determination of GSH level in tissue samples*

Reduced glutathione (GSH) was determined by the method of Ellman (9). Briefly, 1 ml tissue homogenate was treated with 1 ml of 5 trichloroacetic acid (% 10) (Sigma, St. Louis, MO), The mixtures were centrifuged at 5000 rpm and the supernatant was taken.

After deproteinization, the supernatant was allowed to react with 1 ml of Ellman's reagent (30 mM 5, 5'-dithiobisnitro benzoic acid in 100 mL of 0.1% sodium citrate). The absorbance of the yellow product was read at 412 nm in spectrophotometer. Pure GSH was used as standard for establishing the calibration curve.

*Lipid extraction*

Lipid extraction of tissue samples were extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin (10). A tissue sample measuring 1g was homogenized with 10 ml hexane-isopropanol mixture. Fatty acids in the lipid extracts were converted into methyl esters including 2% sulphuric acid (v/v) in methanol (11).

The fatty acid methyl esters were extracted with 5 ml n-hexane. Analysis of fatty acid methyl ester was performed in a Shimadzu GC-17A instrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm i.d.permabond fused-silica capillary column (Macherey-Nagel, Germany). The oven temperature was programmed between 145-215°C, 4°C/min. Injector and FID temperatures were 240 and 280°C, respectively. The rate of

nitrogen carrier gas was at 1 ml/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. *Class GC 10* software version 2.01 was used to process the data. The resulting mg/g tissue was calculated.

*Saponification and extraction*

Alpha-tocopherol and cholesterol were extracted from the lipid extracts by the method of Sanchez-Machado et al. (12) isopropyl alcohol mixture was treated with 5 ml of KOH solution (0.5 M in methanol), which was immediately vortexed for 20 s. The tubes were placed in a water bath at 80°C for 15 min. Then after cooling in iced water, 1 ml of distilled water and 5 ml of hexane was added, and the mixture was rapidly vortexed for 1 min, then centrifuged for 5 min at 5000 rpm. The supernatant phase was transferred to another test tube and dried under nitrogen. The residue was re-dissolved in 1 ml of the HPLC mobile phase (68:28:4 (v/v/v) methanol:acetonitrile:water). Finally, an aliquot of 20 µL was injected into the HPLC column. Before injection, the extracts were maintained at -20°C away from light.

*Total protein assay*

Total protein contents in liver tissue were determined as Lowry's method described. The procedure for measuring protein was followed according to Lowry et al. (13) using BSA (Bovin serum albumin) as standard. The absorbance was read at 750 nm using a spectrophotometer.

*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 (14). 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 ade-nochrome production at 26°C. The solutions used in SOD activity measurement were made fresh daily. The assays were performed 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.

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 contained 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 by using an extinction coefficient of 9.6 mM-1 cm-1 (15).

The decomposition of hydrogen peroxide ($H_2O_2$) was followed directly by the decrease in absorbance at 240 nm and the difference in absorbance per unit time was the measure of catalase activity (16). One unit of catalase activity was defined as the amount of substrate (mmol) consumed in 1 min by 1 mg total protein containing homogenate.

*Statistical analysis*

One-way analysis of variance (ANOVA) and Post Hoc Tukey-HSD test were used to determine differences between groups. Results are presented as mean ±S.E.M. Values were considered statistically significant if p<0.05. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis.

## Results

*Liver Tissue Protein Values*

Total protein levels of liver tissue in Table 1 were found to be significantly reduced in all groups compared to the control group, and this decrease was more pronounced in the DMBA group (p <0.001).

*Liver Tissue MDA Values*

Compared with the control group, it was determined that the MDA level in liver tissue increased significantly in the DMBA group (p <0.001). In the groups containing plant extract, the amount of MDA decreased and this decrease was especially significant in the RC group (p <0.001) (Table 2).

*Liver Tissue GSH Values*

Compared to the control group rats, it was found that the reduced glutathione (GSH) level in liver tissue decreased in all groups and this decrease was more pronounced in the DMBA group rats (p <0.001) (Table 3).

**Table 1.** Liver tissue protein values (mg/g)

| GROUPS | PROTEIN (mg/g) |
|---|---|
| **Control** | 66,77±2,14 |
| **DMBA** | **43,47±2,43**[d] |
| **PT** | 58,55±3,26[b] |
| **PT+DMBA** | **49,10±2,43**[d] |
| **RC** | **51,31±1,37**[d] |
| **RC+DMBA** | **49,96±1,43**[d] |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

**Table 2.** Liver tissue MDA values (nmol/gr)

| GROUPS | MDA (nmol/gr) |
|---|---|
| **Control** | 44,97±0,86 |
| **DMBA** | **61,67±0,56**[d] |
| **PT** | 43,34±0,51[a] |
| **PT+DMBA** | 47,08±0,39[b] |
| **RC** | **29,34±0,36**[d] |
| **RC+DMBA** | 38,55±0,57[b] |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

*Antioxidant Enzymes Activities*

In liver tissue, catalase, glutathione S transferase, superoxide dismutase enzyme levels were examined (Table 4). Catalase enzyme was significantly increased in the DMBA group compared to the control (p <0.01), and the increase in the PT and PT + DMBA groups was more pronounced (p <0.001); and a significant decrease was observed in the RC + DMBA group (p <0.05).

Compared with the control group, the GST enzyme decreased significantly in the DMBA and PT groups (p <0.01). In addition to this, decreased more significantly in the RC group (p <0.001), and the most significantly decreased in the RC + DMBA group (p <0.05); but it significantly increased in the PT + DMBA group (p <0.05).

SOD enzyme activities in liver tissue were calculated as% inhibition and unit and statistical evaluation was done. Compared to control, it was observed that SOD activity increased more significantly in DMBA group (p <0.01), statistically significant increase in RC and RC + DMBA groups (p <0.05), and significantly

**Table 3.** Liver tissue GSH values (µg/gr)

| GROUPS | GSH (µg/gr) |
|---|---|
| **Control** | 447,85±1,30 |
| **DMBA** | **313,11±0,53**[d] |
| **PT** | 431,78±1,64[b] |
| **PT+DMBA** | **411,10±0,76**[c] |
| **RC** | 450,23±0,70[a] |
| **RC+DMBA** | 446,07±0,70[a] |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

decreased in PT group (p <0.01). It was determined that there was no statistical difference in the PT + DMBA group (p> 0.05).

*The A, D, E, K vitamins and cholesterol levels in the liver tissue*

Change of ADEK vitamins, cholesterol and sterol in the liver tissue of the breast cancer-induced rats are indicated in Table 5. It was determined that significant decrease in liver tissue, K1 and stigmasterol levels of DMBA group compared to control (p <0.001). Also, it was observed that significant increase in K2, β -sitosterol and retinol levels (p <0.01) and significant decrease in D2 level (p <0.01). A statistically significant decrease (p <0.05) was observed in δ-tocopherol, D-3, α-tocopherol, ergosterol and cholesterol levels.

Significant reduction in D2, D3, Ergosterol, K1, cholesterol and stigmasterol levels in the PT group compared to the control (p <0.001; p <0.01); a significant increase (p <0.05) was observed in α-tocopherol and β -sitosterol levels, while there was no statistical significance at K2 and retinol levels. Compared to the control group, a significant decrease in δ-tocopherol, D2, D3, K1, cholesterol levels and a significant increase in α-tocopherol, β-sitosterol and retinol levels were observed in the PT + DMBA group (p <0.001; p <0.01). There were no statistical differences in ergosterol and stigmasterol levels (p> 0.05).

It was determined that significant decrease in D-3, K-1, D-2, ergosterol and cholesterol levels in the RC group compared to the control group (p <0.001) and increase in α-tocopherol level (p <0.01). There was no statistical significance in K2, β -sitosterol and retinol levels (p> 0.05).

Compared to the control group, there was a significant increase in α-tocopherol and β-sitosterol levels and a significant decrease in D2, D3, K1, ergosterol and cholesterol levels in the RC + DMBA group (p <0.001; p <0.01).

*Liver Tissue Fatty Acids Values*

Table 6 shows vitamins fatty acis levels in kidney tissue of rats. Compared to control group, a significant increase was observed in 16:0 (Palmitic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linoleic acid), 18:3 (Linolenic acid) and 22:6 n-6 (Docosahexaenoic acid) levels in other groups (p<0,01; p<0.001). It was determined that changes observed in 16:1 n-7 (Palmitoleic acid) and 18:0 (Stearic acid) levels were statistically insignificant (p>0.05).

## Discussion

*Pistacia terebinthus* L. (Menengic) It is one of the Pistacia species belonging to Anacardiaceae family. The plant has different biological activities. It is used as an antiseptic for asthma and bronchitis in various traditional treatments, such as the treatment of burns. Flavonoids such as apigenin, luteolin, luteolin 7-O-glucoside, quercetin and kaempferol have been found in fruit extracts.

**Table 4.** Liver tissue CAT, GST and SOD values

| GROUPS | CAT (µg/g/1dk) | GST (µg/g/1dk) | SOD (% İnhibisyon) | SOD (Unit) |
|---|---|---|---|---|
| **Control** | 1450,76±58,42 | 50,96±3,68 | 26,60±0,98 | 18,16±0,70 |
| **DMBA** | 1596,13±56,28$^{c}$ | 39,62±4,66$^{c}$ | 28,60±1,06$^{c}$ | 20,16±1,02$^{c}$ |
| **PT** | **1750,11±11,83**$^{d}$ | 39,17±5,45$^{c}$ | 24,08±1,07$^{c}$ | 16,41±0,74$^{c}$ |
| **PT+DMBA** | **1704,50±44,56**$^{d}$ | 55,89±2,02$^{b}$ | 26,26±1,02$^{a}$ | 18,14±0,86$^{a}$ |
| **RC** | 1512,40±61,15$^{b}$ | **33,89±5,57**$^{d}$ | 28,15±0,59$^{b}$ | 19,33±0,53$^{b}$ |
| **RC+DMBA** | 1398,17±86,93$^{b}$ | 46,46±3,50$^{b}$ | 27,66±1,01$^{b}$ | 19,42±1,01$^{b}$ |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

**Table 5.** Liver tissue A, D, E, K vitamins and cholesterol values (μg/g)

| Vitamins/Sterol | KONTROL | DMBA | MN | MN+DMBA | SM | SM+DMBA |
|---|---|---|---|---|---|---|
| **K2** | 0,61±0,11 | 1,09±0,25$^{c}$ | 0,66±0,13$^{a}$ | 0,43±0,05$^{b}$ | 0,67±0,05$^{a}$ | 0,72±0,09$^{b}$ |
| **δ-Tokoferol** | 3,10±0,33 | 2,05±0,18$^{b}$ | 2,19±0,36$^{b}$ | **1,61±0,25$^{d}$** | 2,19±0,26$^{b}$ | 3,00±0,27$^{a}$ |
| **D2** | 1,45±0,10 | 0,65±0,32$^{c}$ | **0,24±0,16$^{d}$** | **0,43±0,13$^{d}$** | 0,69±0,11$^{c}$ | **0,48±0,15$^{d}$** |
| **D3** | 2,05±0,16 | 1,71±0,30$^{b}$ | **0,47±0,13$^{d}$** | **0,45±0,14$^{d}$** | **0,26±0,09$^{d}$** | **0,43±0,17$^{d}$** |
| **α -Tokoferol** | 8,79±0,24 | 8,02±1,15$^{b}$ | 9,97±0,85$^{c}$ | **10,76±0,97$^{d}$** | 10,15±2,08$^{c}$ | **10,64±0,99$^{d}$** |
| **Ergosterol** | 6,30±0,57 | 5,76±0,55$^{b}$ | **4,95±0,84$^{d}$** | 6,12±0,54$^{a}$ | 5,34±0,60$^{c}$ | 5,47±0,65$^{c}$ |
| **K1** | 0,35±0,20 | **0,13±0,09$^{d}$** | **0,06±0,03$^{d}$** | **0,08±0,04$^{d}$** | **0,11±0,07$^{d}$** | **0,15±0,05$^{d}$** |
| **Kolesterol** | 563,75±23,09 | 493,63±20,00$^{b}$ | 473,05±27,48$^{c}$ | 470,20±19,22$^{c}$ | **442,40±17,38$^{d}$** | 469,18±9,98$^{c}$ |
| **Stigmasterol** | 173,48±17,93 | **94,35±3,05$^{d}$** | **96,44±14,19$^{d}$** | 164,93±39,88$^{a}$ | 149,42±23,90$^{b}$ | 145,61±11,19$^{b}$ |
| **β -sitosterol** | 2,75±0,31 | 5,36±1,22$^{c}$ | 3,63±0,90$^{b}$ | **6,89±1,77$^{d}$** | 2,92±0,67$^{a}$ | 5,15±0,71$^{c}$ |
| **Retinol** | 102,63±7,65 | 132,27±7,44$^{c}$ | 101,23±11,03$^{a}$ | 135,42±7,05$^{c}$ | 98,05±9,29$^{a}$ | 122,80±6,00$^{b}$ |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

It has been reported to have high antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties due to the richness of secondary compounds in fruits and resins (6). *P. terebinthus* has been found to have a high antioxidant capacity, eliminating free radicals, showing a possible protective role in cancer risk. HPLC characterization of the crude extract of the plant showed that it is rich in compounds such as luteolin and luteolin-7-glucoside. These secondary metabolites have been reported to exhibit high antioxidant activity and have anticarcinogenic properties (5).

*Rhus coriaria*, commonly known as sumac, is a flowering shrub that belongs to the Anacardiaceae family. Many studies have associated with the accumulation of ROS (Reactive oxygen species) in the body, atherosclerosis (17), insulin resistance, type II diabetes (18), cardiovascular diseases (19), osteoarthritis (20) revealed that *R. coriaria* extract was effective on all

**Table 6.** Tissue Fatty Acids Values (%)

| Fatty acids | CONTROL | DMBA | PT | PT+DMBA | RC | RC+DMBA |
|---|---|---|---|---|---|---|
| **16:0** | 13,43±0,27 | 14,66±0,25$^{c}$ | 13,92±0,17$^{a}$ | 14,89±0,38$^{c}$ | 13,17±0,28$^{a}$ | 13,94±0,29$^{a}$ |
| **18:0** | 22,68±0,37 | 18,11±2,66$^{b}$ | 20,56±0,51$^{a}$ | 21,25±0,52$^{a}$ | 22,38±0,26$^{a}$ | 21,56±0,38$^{a}$ |
| **∑SFA** | 36,11±0,10 | 32,77±0,43$^{b}$ | 34,48±0,11$^{a}$ | 36,14±0,14$^{a}$ | 35,55±0,10$^{a}$ | 35,50±0,11$^{a}$ |
| **16:1, n-7** | 1,00±0,05 | 1,03±0,04$^{a}$ | 1,12±0,03$^{b}$ | 1,11±0,04$^{b}$ | 1,05±0,02$^{a}$ | 1,04±0,03$^{a}$ |
| **18:1, n-9** | 3,44±0,19 | 11,12±2,80$^{c}$ | 3,67±0,19$^{a}$ | 11,97±2,94$^{c}$ | 12,71±4,07$^{c}$ | **17,09±2,40$^{d}$** |
| **∑MUFA** | 4.44±0,08 | 12,15±0,49$^{c}$ | 4,79±0,06$^{a}$ | 13,08±0,5$^{c}$ | 17,78±0,75$^{c}$ | **21,59±0,42$^{d}$** |
| **18:2, n-6** | 14,78±0,46 | 14,77±0,57$^{a}$ | 15,00±0,50$^{b}$ | 13,56±0,40$^{c}$ | 14,69±0,63$^{a}$ | 13,62±0,40$^{c}$ |
| **18:3, n-6** | 0,20±0,01 | 0,21±0,01$^{a}$ | 0,23±0,01$^{b}$ | 0,21±0,00$^{a}$ | 0,25±0,01$^{b}$ | **15,57±0,34$^{d}$** |
| **22:2, n-6** | 0,42±0,01 | 0,36±0,03$^{b}$ | 0,44±0,17$^{b}$ | **0,19±0,02$^{d}$** | **0,16±0,03$^{d}$** | 0,22±0,02$^{c}$ |
| **22:6, n-3** | 8,33±0,44 | 9,29±0,28$^{c}$ | 8,49±0,36$^{b}$ | **9,61±0,37$^{d}$** | 8,31±0,39$^{a}$ | **9,62±0,25$^{d}$** |
| **∑PUFA** | 23,73±0,10 | 24,63±0,12$^{a}$ | 24,16±0,11$^{a}$ | 23,57±0,10$^{a}$ | 23,41±0,14$^{a}$ | **39,03±1,76$^{d}$** |
| **∑USFA** | 28,17±0,09 | 36,78±0,30$^{c}$ | 28,95±0,08$^{a}$ | 36,65±0,31$^{c}$ | 41,19±0,44$^{c}$ | **60,62±1,09$^{d}$** |

*d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05*

different diseases such as hepatocyte toxicity (21) and DNA damage (22).

This study examines the effects of *Pistacia terebinthus* subsp. *palaestina* (terebinth), which exhibits high antioxidant activity due to its secondary metabolites, and effects of *Rhus coriaria* (sumac), which has a chemopreventive treatment potential with its strong anti-breast cancer activity, on biochemical parameters in liver tissue in DMBA-induced breast cancer model in rats.

When liver tissue total protein levels are examined, a significant decrease was found in DMBA group compared to the control group ($p<0.001$); in addition, significant reductions were also found in most plant groups. ($p<0.001$). Özdemir et al. (23) investigated the regulatory effects of organoselenium compounds against DMBA-induced biochemical changes in the blood of rats. They reported that DMBA administration causes a decrease in total protein, albumin and globulin levels, while organoselenium compounds, on the other hand, significantly increase total protein and albumin levels. El Kholy et al. (24) investigated the healing effects of hops (*humulus lupulus*), rosemary (*Salvia rosmarinus*) and cat's claw (*Uncaria tomentosa*) plants against 7,12-DMBA-induced hepatic toxicity. They found a significant decrease in total protein, total albumin, globulin and liver total protein levels in the DMBA group. They reported that in addition to serum albumin and globulin, there was a significant improvement in serum and hepatic total protein levels in DMBA-induced rats by supplementing these plants.

In our study, MDA levels in liver tissue were measured spectrophotometrically. Compared with the control group, it was determined that the MDA level in liver tissue increased significantly in the DMBA group ($p <0.001$). In the groups containing plant extract, the amount of MDA decreased and this decrease was especially significant in the RC group Circulating malondialdehyde was reported to be in higher amounts in advanced-stage breast cancer than early breast cancer (24). Malondialdehyde, a product of peroxidation and a marker of oxidative stress, was found to be in higher levels in breast cancer patients compared to the patients in the control group (25).

In our results, the increase in MDA level, a marker of lipid peroxidation, in liver tissue compared to the control group in DMBA groups is related to oxidative stress. Oxidative stress plays a role in the onset and progression of breast cancer (26). However, the decrease of MDA level in antioxidant groups than the control group in our results shows that *P. terebinthus* and *R. coriaria* play a role in cellular defense against oxidative stress.

In our study, reduced glutathione (GSH) levels were measured spectrophotometrically. The results of the present study revealed that the level of GSH in DMBA group was lower than the

control group. However, GSH level was high in the plant extract groups. GSH is the most prevalent low molecular weight antioxidant within cells and protects cellular constituents from oxidative damage by reacting directly with oxidants or acting as the substrate for glutathione peroxidase to scavenge peroxides (27). When the GSH molecule neutralizes the free radicals,

the GSH molecule is converted to oxide form (GSSG). The GSSG is again converted to GSH use to NADPH by the GSH reductase enzyme. The conservation and formation of NADPH in the cells are realized by the activity of pentose-phosphate pathway and malic enzyme (28). In this context, the decrease of GSH levels in cancer groups is an indication of the onset of oxidative stress.

Oxidative stress results from imbalances between antioxidants and free radicals (superoxide anion, hydrogen peroxide, reactive nitrogen types) (26). Various studies have been conducted on the relationship between oxidative stress and human breast cancer. Oxidative stress plays a role in the onset and progression of breast cancer (26). Enzymatic and non-enzymatic defense systems (SOD, CAT, GST and GSH) from endogenous antioxidants prevent or limit tissue damage by removing free radicals in cells (29,30). This study revealed that different amounts of these antioxidant enzymes changes in liver tissue. Also, it has been concluded that the increase or decrease of enzymes may be related to the detoxification mechanisms of the liver.

Compared to the control group, lipophilic vitamins and cholesterol level significantly changed in the plant groups. Lipophilic vitamins are necessary to maintain the physiological functions of the organism, support immune and growth activity and also undertake an important role in stimulating digestion

and synthesis. In addition, several studies tend to concurthat vitamin deficiency increases in some diseases (31). Many hypotheses have suggested that there is a relationship between the occurrence of breast cancer in both animal models and cell lines and vitamin D concentrations in the body (32). High levels of 25-hydroxyvitamin D provide a significant reduction in the postmenopausal incidence of breast cancer (33). In addition, it has been determined with the Vitamin E diet that it is possible to prevent breast cancer and its complications (34).

The polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subject to free radical-initiated oxidation and contribute in chain reactions that amplify damage to biomolecules. Liver tissue is rich in cholesterol. In the present study the level of cholesterol in the liver tissue of cancerous rats decreased compared to the control group.

Lipids are molecules with a wide variety of structural features, including various cytological and pathological processes, such as breast cancer. In tumors, most lipids are secondary indicators and this makes the lipids therapeutic targets and diagnostic biomolecules that support the pathophysiology of the disease. Changes in lipid metabolism in cancer cells can be diagnosed in the early stages of malignancy (35).

It is believed that the increase of lipogenesis, which enables rapid growth of cancer cells, is a characteristic metabolic feature of many cancer types (36). The relationship between plasma lipids (such as total cholesterol, high-density lipoprotein and triglycerides) and breast cancer risk has also been reported in several studies (36-38).

A study on the lipid profile with the carcinogenesis of human breast cancer revealed that Omega-6 polyunsaturated fatty acids (n-6 PUFA) stimulate breast tumor progression and metastasis, while long-chain n-3 polyunsaturated fatty acids exhibit suppressive effects. Besides, the ratio of n-6 to n-3 fatty acids was seen as an important factor in controlling tumor progression. The addition of n-3 PUFA to the diet can alter breast cancer risk factors. Experimental data show that, when added to the culture medium or animal diets, fish oil n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) can suppress tumor cell proliferation and induce apoptosis by multiple mechanisms (39).

It is a known fact that bioactive lipids produced from dietary fat play a role in tumor progression and increase proliferation in cancer cells. Previous studies have shown that dietary polyunsaturated fatty acids, particularly arachidonic acid, produced by eicosanoids, support tumor progression (38,39). Arachidonic acid (20:4 n-6), an essential fatty acid, is suggested to play an important role in the progression of various cancers, including breast cancer.

## Conclusion

The results of the present study showed that the herb suspensions exerted anti-cancer effects and consequently may alleviative liver damage caused by DMBA-induced breast cancer. However, it was observed that they were not sufficiently effective on especially enzyme activities at molecular level. As a result of the obtained results, it is concluded that these plants can be used for cancer patient follow-up and recovery process. This will be important as an indication of the potentially medicinal and economical utility of *P. terebinthus* and *R. coriaria* as new sources of bioactive phytochemicals and functional foods.

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