Turkish Journal of Science & Technology
Volume 9(1),7-16, 2014

Effects of *Rubus discolor* Flower Extract on the Fatty Acid Composition in Hydrogen Peroxide Administered Wistar Rats

[*]S. KESER[1], S. CELIK[2], S. TURKOGLU[3], O. YILMAZ[4], I. TURKOGLU[5]

[1] Firat University, Faculty of Science, Chemistry Department 23119-Elazig/Turkey.
[2] Usak University, Faculty of Science and Arts 64000-Usak/Turkey.
[3] Tunceli University, Faculty of Engineering, Food Engineering Department 62000-Tunceli/Turkey.
[4] Firat University, Faculty of Science, Biology Department 23119-Elazig/Turkey.
[5] Firat University, Education Faculty, Department of Biology Education 23119-Elazig/Turkey.
*Corresponding Author: Dr. Serhat KESER serhatkeser@gmail.com

(Received: 12.08.2013; Accepted: 21.02.2014)

Abstract

The aim of this research is to examine possible protective effects of *Rubus discolor* (RD) flower extracts on the fatty acid contents of some tissues, which hydrogen peroxide (HP) administered Wistar rats. In present study, Wistar rats were randomly divided into four groups: 1. Control (C), 2. Hydrogen Peroxide (HP), 3. *Rubus discolor* (RD), 4. *Rubus discolor* + Hydrogen Peroxide (RD+HP). In tissues, fatty acid content analyses were performed by gas chromatography. According to our results, while fatty acid levels were barely affected in the heart, muscle, brain and spleen tissues of HP group, but these levels were affected in the serum, liver, kidney and lung tissues of the same group when compared to the control group. The fatty acid contents were changed in all tissues of RD and RD+HP groups when compared to control group. Our results confirm that RD and HP treatment have affected the amount of important fatty acids, which substrates in fatty acids metabolism of Wistar rats. In also, this study is first report about the effects of *R. discolor* flower extract on the fatty acid composition.

Key words: *Rubus discolor*, hydrogen peroxide, Wistar rat, fatty acid.

Rubus discolor Çiçek Ekstresinin Hidrojen Peroksit Uygulanmış Wistar Sıçanların Yağ Asidi Kompozisyonu Üzerindeki Etkileri

Özet

Bu çalışmanın amacı hidrojen peroksit (HP) uygulanmış Wistar sıçanların bazı dokularının yağ asidi içeriği üzerinde *Rubus discolor* (RD) çiçek ekstresinin olası koruyucu etkilerini incelemektir. Sunulan çalışmada, Wistar sıçanlar rastgele 4 ayrı gruba ayrıldı: 1. Kontrol (C), 2. Hidrojen Peroksit (HP), 3. *Rubus discolor* (RD), 4. *Rubus discolor* + Hidrojen Peroksit (RD+HP). Dokulardaki yağ asidi içeriği gaz kromatografisi cihazıyla analiz edildi. Sonuçlarımıza göre, kontrol grubuyla karşılaştırıldığı zaman, HP grubunun kalp, kas, beyin ve dalak dokusunda yağ asidi seviyeleri hemen hemen hiç etkilenmezken, aynı grubun serum, karaciğer, böbrek ve akciğer dokularında yağ asitleri seviyeleri etkilenmiştir. RD ve RD+HP gruplarının bütün dokularında yağ asidi içeriği kontrol grubuna göre değişmiştir. Sonuçlarımız göstermiştir ki RD ve HP uygulaması, Wistar sıçanların yağ asidi metabolizmasında substrat olan bazı önemli yağ asitlerinin miktarını etkilemiştir. Ayrıca bu çalışma *R. discolor* çiçek ekstresinin yağ asidi içeriği üzerindeki etkileri hakkında ilk rapordur.

Anahtar Sözcükler: *Rubus discolor*, hidrojen peroksit, Wistar sıçan, yağ asidi

1. Introduction

Rubus species have been cultivated for centuries for their fruits. These and other parts of the plants have been used traditionally for therapeutic purposes [1]. They are characterized by their capability of synthesizing and accumulating ellagitannins, a major class of phenolic compounds largely responsible for the astringent and antioxidant properties of raspberries and blackberries [2,3]. They have also been found to metabolize several phenolic carboxylic acids, such as ellagic acid, and phenyl propenoids, particularly caffeic acid. *Rubus* species have been used in traditional medicine as

antimicrobials, anticonvulsants and muscle relaxants [4,5].

The consumption of fruits and their derived products may prevent diseases related to the oxidative processes due to the large amount of antioxidants present in these foods, especially vitamin C, carotenoids, selenium and polyphenolic compounds [6]. The *Rubus* spp. is an excellent source of polyphenolic compounds with a high amount of anthocyanins and good supply of phenolic acids, flavonoids and other non-anthocyanin flavonoids [7]. A considerable body of research has focused on the antioxidant capacity of the same and their by-products in systems *in vivo* [8].

Diets rich in fruits and vegetables protect against various chronic diseases such as cardiovascular diseases, stroke, and cancers. These beneficial effects have been attributed to high concentrations of antioxidants such as vitamin C, vitamin E, carotenoids, and flavonoids [9-13]. Several epidemiological studies have reported that vitamin supplements or a high intake of fruits such as strawberries or blueberries increase plasma antioxidant capacity and lowers the risk for cardiovascular diseases [10,14,15]. However, other studies have shown that antioxidant supplements or a high intake of vegetables or fruits did not improve antioxidant capacity in healthy subjects. These results suggest that different types of supplements or antioxidants could differentially affect the antioxidant system in humans [16-18].

Reactive oxygen species (ROS) and its metabolites is the subject of intense research because of their active role in cellular physiology and pathogenesis of a number of diseases [19]. ROS also lead to oxidative damage [20]. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids, and its occurrence in biological membranes causes impaired membrane fluidity and inactivation of a several membrane bound enzyme [21]. Until relatively recently, the reactive oxygen species hydrogen peroxide (H_2O_2) was viewed mainly as a toxic cellular metabolite. However, it is now clear that it is much more than that, and functions as a signaling molecule that mediates responses to the various stimuli in both plant and animal cells [22-24]. The generation of H_2O_2 is increased in response to the various stresses, implicating it as a key factor mediating the phenomena of acclimation and cross-tolerance, in which previous exposure to one stress can induce tolerance of subsequent exposure to the same or different stresses [22,25].

In this paper, we aimed to *R. discolor* flower extract possible protective effects on the fatty acid composition against hydrogen peroxide administered Wistar rats due to above mentioned studies have noted *Rubus* species which it contain antioxidant compounds. In also, hydrogen peroxide is a toxic cellular metabolite and it can be caused oxidative stress in the living organisms. In this study, we tried to examine and evaluate the overall impact of all tissues in the *R. discolor* flower extract given and hydrogen peroxide administered rats. To achieve this aim, rats were given *R. discolor* flower water extract by oral gavage for ten weeks, then fatty acid levels in their serum, liver, kidney, muscle, heart, brain, lung and spleen tissues were assessed.

2. Material and Methods

2.1. Chemicals

Hexane, methanol and acetonitrile were obtained from Sigma Chemical Co. (USA). Isopropyl alcohol and sulfuric acid were obtained from Fluka BioChemica (Switzerland). KCl and H_2O_2 were obtained from Merck (Germany).

2.2. Animals

Rats (Wistar albino male) 4 months of age with an average weighing 200–250 g were provided from the Experimental Animal Research Center, Firat University, and were housed in four groups, and each group contained ten rats. The animals were housed at 20±2 °C in a daily light/dark cycle. All animals were fed a group wheat-soybean meal-based diet and water ad libitum in stainless cages, and received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institutes of Health. The ethical regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare

during experiments. This study was approved by The Ethic Committee of Firat University. These treatments were continued for ten weeks, after this process each experimental rat was decapitated, and tissue samples were collected and stored in -85 °C prior to biochemical analysis [26].

2.3. Experimental Design

The rats were randomly divided into four groups each containing ten rats. Group C (Control): the rats received tab water and fed with standard pellet diet as ad libitum. Group HP (Hydrogen Peroxide): to the rats injected intraperitoneally hydrogen peroxide 20 mg/kg in the physiologic saline buffer two times per week and fed with standard pellet diet as ad libitum. Group RD (*Rubus discolor*): the rats received 250 mg/kg *R. discolor* flower water extract orally by gavage four times per week and fed with standard pellet diet as ad libitum. Group RD+HP (*Rubus discolor* + Hydrogen Peroxide): to the rats injected intraperitoneally hydrogen peroxide 20 mg/kg in the physiologic saline buffer two times per week, and the rats received 250 mg/kg *R. discolor* flower water extract orally by gavage four times per week and fed with standard pellet diet as ad libitum. The dose of hydrogen peroxide was determined according to previous studies [27]. At the end of study, serum, liver, kidney, heart, muscle, brain, spleen and lung tissue samples were collected from experimental rats and they stored at -85 °C until to fatty acid analyses.

2.4. Lipid Extraction

Total lipids were extracted with hexane/isopropyl alcohol (3:2 v/v) by the method of Hara and Radin [28]. 1 g tissue sample was homogenized and mixed with 5 mL hexane-isopropyl alcohol (3:2, v/v) in a mixer. Non-lipid contaminants in lipid extracts were extracted into 0.88% KCl solution. The extracts were evaporated in a rotary evaporator flask, and then stored at -25 °C.

2.5. Fatty Acid Analysis

Fatty acids in the lipid extracts were converted into methyl esters, including 2% sulfuric acid (v/v) in methanol [29]. The fatty acid methyl esters were extracted three times with n-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 software computing recorder. Chromatography was performed with a capillary column (25 m in length) and 0.25 mm in diameter, Permabound 25, Machery-Nagel, Germany using nitrogen as a carrier gas (flow rate 0.8 mL/min). The temperatures of the column, detector and injection valve were 130–220, 240, 280 °C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

2.6. Statistical Analysis

The experimental results were reported as mean ± S.E. Statistical analysis was performed using SPSS 15.0 software. Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls.

3. Results

Once the effects of *R. discolor* flower extracts and hydrogen peroxide on the fatty acid composition of Wistar rats were investigated; in kidney tissue, palmitic acid (16:0) and palmitoleic acid (16:1) levels were decreased in all the groups, oleic acid (18:1) and linoleic acid (18:2) levels were increased in the HP group, their levels were decreased in the RD and RD+HP groups when compared to C group. It was observed that stearic acid (18:0), arachidonic acid (20:4) and docosahexaenoic acid (22:6) levels were not differing among all groups in the same tissue. In liver tissue, it was determined that palmitic acid (16:0) and docosahexaenoic acid (22:6) levels were increased in the HP group, but their levels were not differing in the other groups. Palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1) and arachidonic acid (20:4) levels were decreased in all the groups. In brain tissue, palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1), arachidonic acid (20:4) and docosahexaenoic

acid (22:6) levels were increased in the RD+HP group, stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) levels were decreased in the RD group. In muscle tissue, palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) levels were increased in the RD and RD+HP groups, but their levels were not differing in the HP group when compared to the C group. In heart tissue, while linoleic acid (18:2) level was increased in the HP group, docosahexaenoic acid (22:6) level was decreased in the RD and RD+HP groups. In spleen tissue, palmitic acid (16:0) and arachidonic acid (20:4) levels were decreased in the RD and RD+HP groups, docosahexaenoic acid (22:6) level was increased in the same groups. Stearic acid (18:0) and linoleic acid (18:2) levels were decreased in the RD+HP group. In lung tissue, palmitoleic acid (16:1) and linoleic acid (18:2) levels were decreased in all the groups, stearic acid (18:0) level was decreased in the HP group. In serum, it was observed that palmitic acid (16:0) level was increased in the RD and RD+HP groups, and stearic acid (18:0) level was increased in the RD+HP group. Oleic acid (18:1) and linoleic acid (18:2) levels were increased in all the groups, arachidonic acid (20:4) and docosahexaenoic acid (22:6) levels were decreased in the same groups.

Table 1. The fatty acids composition of serum lipids (nmol/mL)

Fatty Acids	Control	HP	RD	RD+HP
16:0	132.54±1.24	134.44±2.04	**143.08±2.88^{b}**	**177.87±1.82^{c}**
16:1	13.44±0.87	**10.69±0.41^{c}**	**21.49±0.41^{c}**	13.90±0.50
18:0	128.83±2.43	136.05±0.46	135.80±3.26	**165.48±3.47^{c}**
18:1	11.10±0.30	**16.86±0.58^{c}**	**25.07±0.69^{c}**	**16.15±0.70^{c}**
18:2	96.71±3.73	**115.85±1.13^{c}**	**144.68±0.84^{c}**	**145.80±2.07^{c}**
20:4	26.74±1.17	**22.14±0.57^{c}**	**17.36±0.33^{c}**	**12.73±0.42^{c}**
22:6	53.26±1.02	**36.95±1.01^{c}**	**42.47±1.77^{c}**	**42.27±0.67^{c}**

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 2. The fatty acid composition of liver lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	1.53±0.02	**1.75±0.07^{b}**	1.52±0.03	1.47±0.03
16:1	0.099±0.009	0.088±0.005	**0.032±0.002^{c}**	**0.034±0.003^{c}**
18:0	1.72±0.09	**1.52±0.07^{a}**	**1.51±0.04^{a}**	1.58±0.04
18:1	0.593±0.020	**0.535±0.012^{a}**	**0.511±0.015^{c}**	**0.525±0.008^{b}**
18:2	1.39±0.08	1.31±0.05	1.53±0.02	1.30±0.04
20:4	4.54±0.21	**3.94±0.23^{a}**	**4.06±0.11^{a}**	**3.88±0.11^{b}**
22:6	0.793±0.068	**0.973±0.043^{b}**	0.758±0.029	0.777±0.030
Others	0.188±0.021	0.250±0.024	0.159±0.020	0.135±0.016
MUFA	0.693±0.026	**0.626±0.012^{a}**	**0.548±0.017^{c}**	**0.562±0.010^{c}**
PUFA	6.62±0.20	6.23±0.30	6.35±0.14	**5.95±0.17^{a}**
Total FA	10.80±0.31	10.39±0.42	10.11±0.22	**9.72±0.20^{a}**

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 3. The fatty acid composition of kidney lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	2.31±0.09	2.43±0.15	**1.55±0.07^{c}**	**1.57±0.05^{c}**
16:1	0.327±0.028	**0.243±0.013^{b}**	**0.112±0.011^{c}**	**0.086±0.008^{c}**
18:0	1.25±0.06	1.17±0.02	1.17±0.05	1.18±0.04
18:1	1.376±0.054	**1.724±0.214^{a}**	**0.808±0.056^{c}**	**0.901±0.029^{b}**
18:2	1.98±0.12	**2.57±0.10^{b}**	**1.52±0.13^{a}**	**1.59±0.08^{a}**
20:4	3.96±0.18	3.67±0.25	4.12±0.18	3.76±0.25
22:6	0.206±0.004	0.240±0.022	0.245±0.014	0.206±0.020

Others	0.563±0.021	0.630±0.023	0.551±0.104	0.304±0.041
MUFA	1.704±0.079	1.968±0.209	**1.014±0.075[c]**	**0.988±0.034[c]**
PUFA	6.15±0.16	6.48±0.23	5.89±0.30	5.56±0.33
Total FA	12.48±0.54	12.53±0.23	**10.19±0.52[b]**	**9.61±0.45[c]**

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 4. The fatty acid composition of heart lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	0.956±0.056	1.144±0.113	0.949±0.029	0.877±0.062
16:1	0.072±0.008	0.088±0.010	0.083±0.003	0.072±0.004
18:0	0.908±0.065	1.007±0.029	1.006±0.040	0.787±0.040
18:1	0.703±0.048	0.788±0.109	0.619±0.031	0.652±0.063
18:2	1.62±0.14	**2.26±0.18[b]**	1.86±0.10	1.79±0.15
20:4	1.99±0.07	2.25±0.11	1.78±0.16	1.69±0.07
22:6	0.773±0.046	0.725±0.079	**0.549±0.063[a]**	**0.582±0.039[a]**
Others	0.678±0.116	0.557±0.065	0.484±0.035	0.411±0.027
MUFA	0.776±0.051	0.876±0.118	0.703±0.034	0.725±0.067
PUFA	4.39±0.20	**5.24±0.11[a]**	4.20±0.28	4.07±0.20
Total FA	7.71±0.30	8.83±0.30	7.35±0.35	6.87±0.35

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 5. The fatty acids composition of muscle lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	0.732±0.087	1.042±0.127	**1.108±0.141[a]**	**1.046±0.068[a]**
16:1	0.087±0.017	0.084±0.019	0.145±0.029	0.085±0.011
18:0	0.309±0.041	0.403±0.037	0.337±0.029	0.386±0.018
18:1	0.403±0.056	**0.676±0.120[a]**	**0.792±0.091[b]**	**0.746±0.089[a]**
18:2	0.886±0.128	1.289±0.166	**1.536±0.121[b]**	**1.514±0.155[b]**
20:4	0.795±0.100	0.798±0.063	0.747±0.030	0.869±0.035
22:6	0.477±0.079	0.506±0.057	0.403±0.011	0.513±0.023
Others	0.254±0.047	0.269±0.051	0.124±0.015	0.316±0.038
MUFA	0.491±0.072	0.761±0.139	**0.938±0.107[b]**	**0.833±0.098[a]**
PUFA	2.16±0.26	2.59±0.26	2.68±0.15	**2.89±0.15[a]**
Total FA	3.94±0.49	5.07±0.61	5.19±0.41	**5.47±0.35[a]**

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 6. The fatty acid composition of brain lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	2.17±0.13	2.04±0.12	1.94±0.04	**2.48±0.10[a]**
16:1	0.063±0.004	0.072±0.004	0.068±0.005	**0.123±0.020[b]**
18:0	2.25±0.14	2.13±0.08	**1.86±0.06[a]**	2.49±0.10
18:1	2.49±0.09	2.57±0.09	2.23±0.07	**3.02±0.09[c]**
18:2	0.170±0.029	0.171±0.024	**0.109±0.011[a]**	0.164±0.011
20:4	1.96±0.10	1.95±0.06	1.74±0.05	**2.30±0.09[b]**
22:6	3.57±0.27	3.58±0.07	3.20±0.06	**4.00±0.13[a]**
Others	1.89±0.12	1.71±0.09	1.30±0.03	2.13±0.06
MUFA	2.55±0.09	2.64±0.10	2.30±0.07	**3.15±0.10[c]**
PUFA	5.72±0.36	5.70±0.12	**5.06±0.12[a]**	**6.48±0.20[a]**
Total FA	14.64±0.66	14.41±0.44	**12.49±0.30[b]**	**16.74±0.45[b]**

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 7. The fatty acid composition of spleen lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	2.04±0.13	1.81±0.16	**1.53±0.16[a]**	**1.10±0.08[c]**

16:1	0.283±0.050	0.215±0.021	0.336±0.053	0.396±0.057
18:0	1.19±0.07	1.16±0.07	1.02±0.10	**0.78±0.04[b]**
18:1	1.092±0.126	0.862±0.103	0.843±0.068	0.915±0.148
18:2	1.23±0.11	1.07±0.15	1.39±0.17	**0.63±0.18[a]**
20:4	1.86±0.10	1.74±0.13	**1.32±0.22[a]**	**0.78±0.04[c]**
22:6	0.108±0.011	0.125±0.019	**0.262±0.050[b]**	**0.430±0.025[c]**
Others	0.504±0.256	0.376±0.050	1.982±0.552	3.342±0.402
MUFA	1.38±0.17	1.08±0.12	1.16±0.09	1.28±0.21
PUFA	3.20±0.16	2.91±0.30	3.00±0.18	**1.84±0.20[c]**
Total FA	8.33±0.49	7.33±0.70	8.75±0.59	8.44±0.86

a: P<0.05 **b:** P<0.01 **c:** P<0.001

Table 8. The fatty acid composition of lung lipids (mg/g)

Fatty Acids	Control	HP	RD	RD+HP
16:0	1.25±0.12	1.03±0.04	1.29±0.08	1.17±0.04
16:1	0.855±0.004	**0.608±0.021[b]**	**0.692±0.050[a]**	**0.642±0.026[b]**
18:0	0.507±0.038	**0.407±0.018[b]**	0.500±0.014	0.512±0.021
18:1	0.968±0.103	0.721±0.031	0.848±0.108	0.767±0.030
18:2	1.613±0.352	**0.918±0.032[b]**	**1.013±0.102[a]**	**0.559±0.074[c]**
20:4	0.448±0.029	0.384±0.017	0.471±0.010	0.498±0.025
22:6	0.323±0.031	0.244±0.016	0.348±0.022	0.371±0.030
Others	4.62±0.59	4.00±0.17	5.21±0.37	4.36±0.26
MUFA	1.82±0.18	**1.33±0.05[a]**	1.54±0.15	**1.41±0.05[a]**
PUFA	2.40±0.38	**1.54±0.06[b]**	**1.83±0.11[a]**	**1.43±0.08[c]**
Total FA	10.60±1.26	**8.30±0.32[a]**	10.39±0.69	8.89±0.33

a: P<0.05 **b:** P<0.01 **c:** P<0.001

4. Discussion

According to these results, it was determined that administration of hydrogen peroxide has not affected fatty acid levels in the HP groups, but their levels has changed in the RD and RD+HP groups by intake of *R. discolor* flower extracts. This result can be related with the effects of *R. discolor* flower extract on the fatty acid metabolism of Wistar rats.

In mammals, it is effective two different fatty acid metabolisms. The first of these, is synthesized as *de nove* from carbohydrate and amino acid precursors in the body, and located in tissue phospholipids and storage lipids such as palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), eicosenoic (20:1), docosanoic (22:0) and lingoseric acids (24:0); the latter starting with linoleic (18:2) and linolenic acid (18:3) the essential fatty acid metabolism. The second fatty acid metabolism is known essential fatty acid metabolism in mammals. Because, 18:2 and 18:3 fatty acids were not synthesized as *de nove* due to the absence of Δ12 desaturase and Δ15 desaturase enzymes, especially in the terrestrial mammals, which is required for beginning this metabolism. But they must be taken into the body through dietary. 14:0, 16:0, 18:0 and 18:1 are endogen synthesized fatty acids [30-33].

In this study, while the palmitic acid (16:0) level was increased in the muscle, serum and brain tissues of RD and RD+HP groups, and its level was decreased in the spleen and kidney tissues of the same group. It can be said that RD extract were affected enzyme activities on duty lipid metabolism, such as acetyl CoA carboxylase and fatty acid synthase. Palmitic acid is the final product in the fatty acid metabolism, and it is synthesized by fatty acid synthase *via* some enzymatic reactions. The increasing of palmitic acid level can be explained by the increase in the fatty acid synthesis of the serum, muscle and brain tissues. Accordingly, the decreasing of its level may be attributed to decrease in this synthesis of the spleen and kidney tissues. According to the needs of cells, palmitic acid is converted to palmitoleic acid (16:1) by Δ9 desaturase

(stearoyl CoA desaturase (SCD)) for the synthesis of phospholipids sphingolipids, triglycerides and cholesterol esters, or it is used by elongase for stearic acid (18:0) synthesis. SCD enzyme is used to palmitic acid and stearic acid as substrate, and it catalyzes formation to palmitoleic and oleic acid from palmitic and stearic acid. This enzyme has been determined in the many tissues of mammals. In our study, palmitoleic acid level was increased in the serum and brain tissues of RD and RD+HP groups, its level was decreased in the lung, liver and kidney tissues of the same groups when compared to control group. While the stearic acid level was increased in the serum of RD and RD+HP groups; its level was decreased in the brain, liver and spleen tissues of the same groups. We have been thought that increasing of the serum stearic acid level can be due to increasing of the SCD enzyme activity, and decreasing of the brain, liver and spleen stearic acid levels may be caused decreasing of the same enzyme activity. It has known that SCD activity was affected by some conditions, such as nutrition and hormonal changes. This enzyme is located in the endoplasmic reticulum membrane, and these events occur in the endoplasmic reticulum. The conversion to unsaturated fatty acids (palmitoleic and oleic acid) from saturated fatty acids (palmitic and stearic acid) is very important metabolic pathway for stability in the cell. The synthesis of stearic acid is provided from palmitic acid via chain extension reactions [33]. Elongase enzyme is very important for the protection of cell membrane fluidity, which allows this stability. When the stearic acid level increased, and joining to cell phospholipids or storage with the triglycerides is not provided, these events were caused abnormal changes in the cell. The activities and levels of elongase, fatty acid synthase, SCD can be affected by different nutritional diet, hormonal changes and added foods in the diet. In the RD and RD+HP groups, while the oleic acid level was decreased in the kidney and liver tissues, its level was increased in the serum and muscle tissues when compared to the control group. The changes of this fatty acid level can be explained by changes of SCD enzyme activities in these tissues. SCD used to stearic acid as substrate, and it converts stearic acid to oleic acid [34].

Excessive poly unsaturated fatty acids (linoleic and linolenic acids) cannot be synthesized by mammal and thus, the organism is completely dependent on their dietary intake. This metabolism is called essential fatty acid metabolism, and it begins with linoleic (18:2) and linolenic acid (18:3) in all the tissues. It continues with chain elongation and activation of enzymes that allow entry of a double band to hydrocarbon chain. The mammals cannot be synthesized linoelic and linolenic acids due to they do not have Δ12 and Δ15 desaturase enzymes. The activation of Δ5 and Δ6 desaturase enzymes is provided occurring of long chain and unsaturated fatty acids such as eicosadienoic acid (20:2), eicosatrienoic acid (20:3), arachidonic acid (20:4), docosadienoic acid (22:2), docosatetraenoic acid (22:4), docosapentaenoic acid (22:5) from linoleic acid (18:2) in the cells of mammal organism. By these enzymes as the substrate, the using of linolenic acid (18:3) is occurred steridonic acid (18:4), eicosapentaenoic acid (22:5) and docosahexaenoic acid (22:6). These two metabolic pathways are called Δ6 desaturation pathway, and is occurred chain elongation of fatty acids by these enzyme systems [30-35]. Free radicals are damaged to cells and tissues via attacking to unsaturated fatty acids, sulfhydryl bonds of DNA and protein molecules in the cell [36]. In the present study, it was determined that while the linoleic acid level was decreased in the lung, brain, kidney and spleen tissues, its level was increased in the serum and muscle tissues in the RD and RD+HP groups when compared to control group. It can be said that while Δ5 and Δ6 enzyme activities were increased in the lung, brain, kidney and spleen tissues, these enzymes activities were decreased in the serum and muscle tissues. So, it can be explained that why changed the linoleic acid levels in these tissues.

Arachidonic acid (20:4) is synthesized by Δ6 desaturation pathway from linoleic acid (18:2). In the present study, it was observed that while the arachidonic acid level was increased in the brain tissue, its level was decreased in the serum, liver and spleen tissues in the RD and RD+HP groups when comparison to control group. Especially in the serum, while the linoleic acid level was increased in all the groups, arachidonic acid level was decreased in same groups. This

situation can be explained by decreasing of Δ5 and Δ6 desaturase activities in the serum. In the serum, liver and spleen tissues, these enzyme activities may be increased. We have been thought *R. discolor* flower extract were affected these fatty acid levels due to these changes occurred in the extract given RD and RD+HP groups. In addition, a reduction of linoleic acid and arachidonic acid levels may be related to the synthesis of eicosanoids. Eicosanoids are provided conversion of arachidonic acid to leukotrienes and prostaglandins via lipoxygenase and cyclooxygenase enzymes. A reduction of arachidonic acid level may be explained by activation of this metabolic pathway. It was observed that docosahexaenoic acid level was increased in the brain and spleen tissues, its level was decreased in the serum and heart tissues in the RD and RD+HP groups, which is the other end product of Δ6 desaturation pathway. It has been suggested this fatty acid level was increased in some pathological conditions, and it has been claimed that cause of this increasing is influenced this pathway by factors of various diseases [33,35].

Özşahin [37] have reported that while linoleic acid and arachidonic acid levels were decreased in the kidney tissue, their levels were increased in the serum, liver and brain tissues in the apricot and grape extracts given rats when compared to control rats. Ayerza and Coates [38] have determined that while the linoleic acid level was not changed, arachidonic acid level was decreased in Chia (*Salvia hispanica*) fed rats. Mohamed et al. [39] have suggested that miristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and arachidonic acid (20:4) levels were increased in the coconut-oil fed rats. Çelik and Özkaya [27] have reported that palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) levels were increased; linoleic acid (18:2), arachidonic acid (20:4) and docosahexaenoic acid (22:6) levels were decreased by H_2O_2 administration in the brain of Guinea pigs. In the present study, fatty acid levels were clearly affected by *R. discolor* flower extract in the Wistar rat tissues as studies of these researchers. In this case, we can say that fatty acid levels were changed by plant extracts in the experimental animals.

5. Acknowledgement

This work was supported by TUBITAK, under grand number 107T898 and it was supported by Firat University, under grand number FÜBAP 1936.

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