

## Antioxidant Properties of *Rubus discolor* L. Extracts and Protective Effects of Its Flower Extract Against Hydrogen Peroxide-Induced Oxidative Stress in Wistar Rats

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In the present study, *in vitro* antioxidant activities of ethanol and water extracts of *Rubus discolor* L. leaves, flower, unripe and ripe fruits were investigated. Additionally, the antioxidant and protective properties of *R. discolor* flower (RD) water extract on some important biochemical parameters against hydrogen peroxide (HP)-induced oxidative stress in rats were determined. The phytochemical profiles and antioxidant properties (scavenging against DPPH<sup>•</sup>, ABTS<sup>•+</sup>, H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>, metal chelating, inhibition of lipid peroxidation and reducing power) of extracts were determined by spectrophotometric methods and HPLC. The antioxidant potential of RD flower extract supplementations were evaluated by measuring total protein, glutathione (GSH), malondialdehyde (MDA), vitamin A and E, cholesterol, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) levels in various tissues. The flower extracts were the most active extracts for *in vitro* antioxidant assays. In HP administered groups, MDA level was increased, GSH and total protein levels were decreased. In RD treated group, these substances levels were protected. In RD plus HP treated group, SOD and GSH-Px levels were increased. These results show that RD flower extract have the protective effects against H<sub>2</sub>O<sub>2</sub> via its free radical scavenging activity, and this extract affected the level of antioxidant enzymes, vitamin A and E, cholesterol, GSH, total protein and MDA.

**Key words:** *Rubus discolor*, MDA, GSH, SOD, Hydrogen peroxide, Cholesterol.

### ***Rubus discolor* L. Ekstrelerinin Antioksidan Özellikleri ve Çiçek Ekstresinin Hidrojen Peroksit-Nedenli Oksidatif Stres Oluşturulmuş Wistar Sıçanlarda Koruyucu Etkileri**

Sunulan çalışmada, *Rubus discolor* L. yaprak, çiçek, ham meyve ve olgun meyvelerinin etanol ve sulu ekstrelerinin *in vitro* antioksidan aktiviteleri incelenmiş ve *R. discolor* çiçek su (RD) ekstresinin hidrojen peroksit (HP)-nedenli oksidatif stres oluşturulmuş sıçanlarda bazı önemli biyokimyasal parametreler üzerindeki antioksidan ve koruyucu etkileri saptanmıştır. Ekstrelerin fitokimyasal profili ve antioksidan özellikleri (DPPH<sup>•</sup>, ABTS<sup>•+</sup>, H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup> yok edici, metal şelatlama, lipit peroksidasyonun inhibisyonu ve indirgeme kuvveti aktiviteleri) spektrofotometrik metotlar ve YBSK cihazıyla belirlendi. RD çiçek su ekstresi uygulamasının antioksidan potansiyeli çeşitli dokularda total protein, glutatyon (GSH), malondialdehit (MDA), A ve E vitaminleri, kolesterol, glutatyon peroksidaz (GSH-Px), süperoksit dismutaz (SOD) seviyelerinin ölçülmesiyle değerlendirildi. *In vitro* antioksidan testlerde çiçek ekstrelerinin en aktif ekstreler olduğu belirlendi. HP uygulanan gruplarda, MDA seviyesi artmışken, GSH ve total protein seviyeleri azalmıştır. RD uygulanan grupta ise bu maddelerin seviyeleri korunmuştur. RD + HP uygulanan grupta SOD ve GSH-Px seviyeleri artmıştır. Bu sonuçlar göstermiştir ki RD çiçek ekstresi H<sub>2</sub>O<sub>2</sub>'e karşı serbest radikal yok etme aktivitesi sayesinde koruyucu etkilere sahiptir ve bu ekstre antioksidan enzimlerle, vitamin A ve E, kolesterol, GSH, total protein ve MDA seviyelerini etkilemiştir.

**Anahtar kelimeler:** *Rubus discolor*, MDA, GSH, SOD, Hidrojen peroksit, Kolesterol.

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## INTRODUCTION

*Rubus discolor* L., is a plant belonging to the Rosaceae family, genus *Rubus*. The geographical distribution of *Rubus* covers a wide range from Europe to northern Asia and most temperate areas. *Rubus* species have been cultivated for centuries. *Rubus* species and their fruits have been used traditionally for therapeutic purposes in many countries to treat wounds, colic pain and some other diseases such as diarrhea and renal disease as well as nutritional purposes (1-3). 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 (4,5). They have found to metabolize several phenolic carboxylic acids, such as ellagic acid, and phenyl propenoids, particularly caffeic acid (6,7).

The reactive oxygen species (ROS) play a major role in either the initiation or progression of carcinogenesis by inducing oxidative stress. Peroxides and superoxide anion produce cytotoxicity/genotoxicity in cellular system (8-10). ROS and nitrogen species are formed in the human body, and endogenous antioxidant defenses are not always sufficient to counteract them completely. A large number of studies have been supported the oxidative stress caused the DNA, lipid and protein damages, therefore this fact may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases (11,12). Diet-derived antioxidants may play an important role to prevent the chronic diseases (13). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (14), and it together with superoxide radical anion can damage many cellular components (15). Recently, the hydrogen peroxide was viewed mainly as a toxic cellular metabolite. The generation of hydrogen peroxide 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 (16-19).

In this paper, we evaluated the antioxidant and radical scavenging activities of *R. discolor* flower, leaves, unripe and ripe fruit water and ethanol extracts by several different *in vitro* antioxidant test systems, such as the DPPH, ABTS and superoxide radical scavenging, inhibition of lipid peroxidation, H<sub>2</sub>O<sub>2</sub> scavenging, the reducing power and the metal chelating activity assays. We also investigated the possible protective effects of the *R. discolor* flower water extract against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage in Wistar rats. To achieve this aim, rats were given *R. discolor* flower water extract by oral gavage for ten weeks, then malondialdehyde (MDA), glutathione (GSH) levels, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, retinol, tocopherol, and cholesterol levels in their serum, liver, kidney, muscle, heart, brain, lung and spleen tissues were assessed.

## EXPERIMENTAL

### *Chemicals and standards*

All chemical compounds and standards were obtained from Sigma-Aldrich (Germany).

### *Plant materials and extraction procedures*

*Rubus discolor* L. flowers, leaves, unripe fruits and ripe fruits were collected from Bingol/Turkey in spring and summer of 2011. Voucher specimen number is Turkoglu 4820. All samples were dried in air and at dark. For extraction, 25 g of sample was mixed with 100 mL solvent (water and ethanol). Extraction continued until the extraction solvents became colorless. The obtained extracts were filtered and the filtrate was collected, and then solvent was removed. All extraction processes were repeated three times. The dried real extract and standard antioxidants were dissolved at 100-1000 µg/mL concentration (20).

### *In vitro studies*

*Determination of antioxidant properties and total phenolic compounds of R. discolor extracts*

The spectrophotometric analysis of 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) radical scavenging capacity was determined according to the method of Re et al (21). ABTS<sup>•+</sup> was produced by reacting 2 mM ABTS in H<sub>2</sub>O with 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, and it was stored for 2 h at room temperature in the dark. The ABTS<sup>•+</sup> solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer (pH=7.4). Then, 1 mL of ABTS<sup>•+</sup> solution was added to 3 mL of extracts at 100 µg/mL concentrations. After 0.5 h, absorbance was recorded at 734 nm. The extent of decolourization was calculated as percentage reduction of absorbance.

The 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH<sup>•</sup>) scavenging capacity was measured by using the method of Shimada et al (22). Briefly, 0.1 mM solution of DPPH<sup>•</sup> in ethanol was prepared and 1 mL of this solution was added to 3 mL of extracts solution at 100 µg/mL concentration. Absorbance at 517 nm was measured after 0.5 h against a blank solution containing the ethanol. Lower absorbance of the reaction mixture indicates the higher DPPH radical scavenging activity.

The measurements of superoxide anion scavenging capacity were based on the method described by Liu et al (23) with slight modification. One milliliter of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT in 100 mmol/L phosphate buffer, pH=7.4), 1 mL nicotinamide adenine dinucleotide (NADH) solution (468 mmol/L in 100 mmol/L phosphate buffer, pH=7.4) and 100 µL of sample solution of extracts were mixed. The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 mmol/L PMS in 100 mmol/L phosphate buffer, pH=7.4) to the mixture. The mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture shows the increase in superoxide anion scavenging capacity.

The chelating of ferrous was estimated by the method of Dinis et al (24). Briefly, 100 µg/mL extracts were added to a solution of 2 mM FeCl<sub>2</sub> (0.05 mL). The reaction was started by addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and

then it was kept at room temperature for 10 min. Absorbance of the solution was measured at 562 nm.

The reducing power activities were determined by the method of Oyaizu (25). Briefly, 100 µg/mL of extract in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (TCA) (2.5 mL, 10%) was added to the mixture, and then centrifuged for 10 min at 1000×g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl<sub>3</sub> (0.5 mL, 0.1%) and the absorbance was measured at 700 nm.

The hydrogen peroxide scavenging activity of extracts was determined according to the method of Ruch et al (26). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH=7.4). Extracts in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of extracts was measured 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide at 230 nm.

Inhibition of lipid peroxidation was determined according to the ferric thiocyanate method in linoleic acid emulsion (27). With this method peroxide formation occurred during the oxidation of linoleic acid oxidation.

All test and analyses were repeated three times and average values were calculated. The antioxidant or antiradical activities of samples were estimated by the following equation:

$$\% \text{ Scavenging Activity} = [(A_0 - A_1)/A_0] \times 100$$

where A<sub>0</sub> is the absorbance of control, and A<sub>1</sub> is the absorbance of the sample in the presence of extracts or standards.

#### *Determination of polyphenolic contents*

Total polyphenolic contents in the extracts were determined with Folin–Ciocalteu's reagent according to the method of Slinkard and Singleton (28) using pyrocatechol and quercetin as standard phenolic compounds. Briefly, 1 mg/mL of the extract solution in a volumetric flask was diluted with distilled water (46 mL). One milliliter of Folin–Ciocalteu's reagent was added and the content

of the flask was mixed thoroughly. After 3 min, 3 mL of Na<sub>2</sub>CO<sub>3</sub> (2%) was added and then it was intermittent shaken for 2 h. The absorbance was measured at 760 nm. The total concentrations of phenolic contents in the extracts were determined as milligram of pyrocatechol and quercetin equivalents by using an equation that was obtained from standard pyrocatechol and quercetin calibration curves:

$$\text{Absorbance} = 0.00053 \times \text{total phenols} [\text{quercetin equivalent (mg)}] + 0.00019.$$

$$\text{Absorbance} = 0.00198 \times \text{total phenols} [\text{pyrocatechol equivalent (mg)}] + 0.00158.$$

#### *Chromatographic conditions for flavonoid Analysis*

Chromatographic analysis was carried out using PREVAIL C 18 reversed-phase column (150 x 4.6 mm x 5 µm) diameter particles. The mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (29). This phase was filtered through a 0.45 µm membrane filter (millipore), then deaerated ultrasonically prior to use. Naringin, rutin, apigenin, myricetin, naringenin and quercetin were quantified by DAD following RP-HPLC separation at 280 nm for naringin and naringenin, 254 nm for rutin and myricetin, 306 nm for apigenin and 265 nm for quercetin. Flow rate and injection volume were 1.05 mL/min and 10 µL, respectively. The peaks in the chromatograms of the extracts were confirmed by comparing to their retention time and UV spectra with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at 25 °C.

#### *Animal studies*

##### *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 (WMA, 2000). The ethic 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, Faculty of Medicine. The ethical approval date, certification and decision number are 26.03.2009, 20/29, respectively. 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 (30).

#### *Experimental design*

The rats were randomly divided into four groups each containing ten rats. It was done preliminary experiments before starting to the study for minimize possible hitches.

Group C (Control): The rats received tap water and fed with standard pellet diet as ad libitum.

Group HP (Hydrogen Peroxide): The rats were injected intraperitoneally with hydrogen peroxide at a concentration of 20 mg/kg in 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): The rats were injected intraperitoneally with hydrogen peroxide at a concentration of 20 mg/kg in 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.

Each experimental rat was decapitated one week after the last injection HP and the last intake RD flower extract (31).

#### *Biochemical analysis*

MDA concentration in tissues was determined using the method described by

Jain et al. (32) based on thiobarbituric acid (TBA) reactivity. GSH concentration in tissues was measured using the method described by Beutler et al (33). The total protein amount was determined using the method described by Lowry et al (34). GSH-Px activity was assayed according to Paglia and Valentine (35) based on that of GSH-Px catalyses the oxidation of GSH. SOD activity was measured at 505 nm by calculating inhibition percentage of formazan dye formation (36).

#### *Determination of vitamin A and E levels in tissue samples*

Tissue samples were homogenized in 3 mL acetonitrile/methanol/isopropyl alcohol (2:1:1, v/v/v) containing tubes and the samples were vortexed for 30 s and centrifuged at 6000×g for 10 min at 4 °C. Supernatants were transferred to autosampler vials of the HPLC instrument. For lipophilic vitamins, the mixture of acetonitrile/methanol (3:1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 mL/min. The temperature of column was kept at 40 °C. Supelcosil™ LC 18 DB column (250 x 4.6 mm, 5 µm; Sigma, USA) was used as the HPLC column and detection was performed at 320 nm for retinol (vitamin A), and 215 nm for  $\alpha$ -tocopherol,  $\alpha$ -tocopherol acetate (37). Identification of the individual vitamins was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions. Quantification was carried out by external standardization using Class VP software. The results of analysis were expressed as µg/g tissue (38).

#### *Total cholesterol analysis in tissue samples*

The tissue samples were homogenized in 3 mL acetonitrile/isopropyl alcohol (70:30, v/v)-containing tubes and the mixture were vortexed for 30 s and centrifuged at 6000×g for 10 min at 4 °C. Supernatants were transferred to autosampler vials of the HPLC instrument. Acetonitrile-isopropyl alcohol (70:30 v/v) was used as mobile phase at a flow rate of 1 mL/min (39). Supelcosil LC 18™ DB column (250 x 4.6 mm, 5 µm) was used as the HPLC column. Detection was performed by UV at 202 nm and 40 °C column oven (40). Quantification was carried

out by external standardization using Class VP software. The results were expressed as µg/g wet weight tissue (38).

#### *Statistical analysis*

The experimental results were reported as mean  $\pm$  S.D. 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.

## RESULTS

#### *In vitro studies*

In this study, we firstly evaluated the *in vitro* antioxidant properties of RD leaf, flower, unripe and ripe fruits using the ABTS, DPPH, superoxide, H<sub>2</sub>O<sub>2</sub> scavenging, chelating of ferrous ions, reducing activity, inhibition of lipid peroxidation, determination of total phenolic compounds and flavonoid contents. Table 1 shows ABTS, DPPH, superoxide, H<sub>2</sub>O<sub>2</sub> scavenging, chelating of ferrous ions, reducing activity, inhibition of lipid peroxidation results of RD water and ethanol extracts. The most effective extracts are, flower ethanol extract for ABTS, H<sub>2</sub>O<sub>2</sub> scavenging and reducing power tests; ripe fruit water extract for superoxide scavenging test; flower water extract for metal chelating and DPPH scavenging tests; unripe fruit water extract for inhibition of lipid peroxidation test.

Table 2 shows total phenolic contents of RD water and ethanol extracts as quercetin and pyrocatechol equivalents. 93.58, 152.05, 74.54, 92.75, 69.96, 72.06, 70.85 and 91.08 mg quercetin equivalent of phenols was detected in 1 g of extracts of RD flower water, flower ethanol, leaf water, leaf ethanol, unripe fruit water, unripe fruit ethanol, ripe fruit water and ripe fruit ethanol, respectively. 23.12, 26.47, 20.25, 18.72, 19.30, 18.34, 18.82 and 18.57 mg pyrocatechol equivalent of phenols was detected in 1 g of extracts of RD flower water, flower ethanol, leaf water, leaf ethanol, unripe fruit water, unripe fruit ethanol, ripe fruit water and ripe fruit ethanol, respectively.

Flavonoid contents of RD extracts shown in Table 3 Rutin, apigenin, naringin, naringenin, myricetin and quercetin were determined in the RD extracts. 856, 44, 261, 809, 41 and

2011 µg rutin, apigenin, myricetin, naringin, naringenin and total flavonoid (respectively) were detected in 1 g of extract of RD leaf. 310, 15, 2682, 2962, 22, 5 and 5996 µg rutin, apigenin, myricetin, naringin, naringenin, quercetin and total flavonoid (respectively) were detected in 1 g of extract of RD flower. 336, 8, 86, 238 and 668 µg rutin, apigenin, myricetin, naringin and total flavonoid (respectively) were detected in 1 g of extract of RD unripe fruit.

#### *Animal studies*

Following the exposure of experimental groups; the effects of RD flower extract, hydrogen peroxide and RD plus HP, on oxidative stress and antioxidative role were evaluated as enzymes (SOD and GSH-Px), MDA content, GSH, total protein, cholesterol, vitamin A and E levels of serum, liver, kidney, heart, muscle, brain, lung and spleen tissues samples from control and treated rats (Table 4 - 11).

#### *Malondialdehyde (MDA) levels*

When the HP treated group was compared with the control group at the end of 10<sup>th</sup> week, the MDA levels were significantly increased in kidney, spleen and lung tissues. MDA levels were not changed muscle tissue of HP and control groups, but significantly decreased in liver and brain tissues. In the RD flower extract treated group, the MDA levels were statistically and significantly decreased in the muscle, brain and lung tissues. There is no difference in the MDA levels of liver, kidney, and spleen tissues compared to control group. When the RD flower extract plus HP treated group was compared with the control group, there is a decrease in the MDA levels of liver, kidney, brain, and lung tissues. Additionally an increase in the MDA levels of muscle and spleen tissues was observed (Table 5-11).

#### *Superoxide dismutase (SOD) activity*

In the serum, compared to the control group, there were statistically significantly increase in the SOD activity in the RD treated and RD plus HP treated groups at the end of 10<sup>th</sup> week. However, there was not any difference for SOD activity in the HP treated group compared with the control group (Table 4).

#### *Glutathione peroxidase (GSH-Px) activity*

A significant increase in GSH-Px activity was observed at the end of the 10<sup>th</sup> week in the RD plus HP treated group compared with the control group, but no difference was observed in the serum samples of HP treated and RD treated groups compared with the control group (Table 4).

#### *Triglyceride and lipoprotein levels*

A significant decrease in triglyceride level was observed in the HP treated group compared with the control group, and a significant increase was observed in the RD plus HP treated group in the serum. There was not any difference in triglyceride level in the RD treated group. The RD plus HP treated group significantly increased in the HDL, LDL and VLDL levels in comparison to the control group. Nevertheless, the HDL and LDL levels were not changed statistically in the HP treated and RD treated groups compared to the serum samples of control group. LDL level was increased in the RD treated group, but it was not any difference in the HP treated group compared with the control group (Table 4).

#### *Reduced glutathione (GSH) levels*

When the HP treated group was compared with control group, there were a significant decrease in the liver, kidney, muscle, heart, brain and lung tissues, GSH levels were increased in the spleen tissue. In the RD treated group, GSH levels were increased in the kidney, heart and brain tissues, and there is no statistical difference in the liver and muscle tissues. It was decreased in the spleen and lung tissues compared to control group. When the RD plus HP treated group was compared with the control group, there was a significant decrease in the liver, muscle, brain, spleen and lung tissues, and there was a significant increase in the GSH levels of kidney and heart tissues (Table 4-11).

#### *Total protein levels*

In the liver, kidney, muscle, heart, spleen and lung tissues, total protein levels were significantly decreased in the HP treated group in comparison to the control group. In the brain tissue, there was not any statistically difference in comparison to control group in

**Table 1.** ABTS, superoxide, DPPH scavenging, metal chelating, inhibition of lipid peroxidation, hydrogen peroxide scavenging and reducing power results of *R. discolor* extracts and some standard antioxidants (100 µg/mL)

Samples	% ABTS Scavenging	% Superoxide Scavenging	% Metal Chelating	% DPPH Scavenging	% Inhibition of Peroxidation	% H <sub>2</sub> O <sub>2</sub> Scavenging	Reducing Power
Control	0	0	0	0	0	0	0.027±0.001
Flower water	96.20±0.35 <sup>a</sup>	87.16±0.21 <sup>b</sup>	54.58±0.77 <sup>b</sup>	84.42±0.83 <sup>b</sup>	92.60±0.09 <sup>b</sup>	42.07±0.64 <sup>a</sup>	0.600±0.012
Flower ethanol	96.89±0.43 <sup>a</sup>	82.11±0.94 <sup>c</sup>	52.82±0.88 <sup>b</sup>	81.61±0.31 <sup>b</sup>	90.20±0.12 <sup>b</sup>	98.77±0.05 <sup>b</sup>	1.589±0.052
Leaf water	96.47±0.28 <sup>a</sup>	84.83±0.46 <sup>c</sup>	40.35±0.75 <sup>c</sup>	80.80±0.28 <sup>b</sup>	93.67±0.05 <sup>b</sup>	28.57±0.54 <sup>c</sup>	0.312±0.011
Leaf ethanol	97.30±0.11 <sup>a</sup>	80.33±0.44 <sup>c</sup>	44.94±0.84 <sup>c</sup>	81.34±0.66 <sup>b</sup>	90.41±0.21 <sup>b</sup>	38.69±0.48 <sup>a</sup>	0.444±0.018
Unripe fruit water	60.17±1.58 <sup>c</sup>	82.50±0.81 <sup>c</sup>	48.35±1.05 <sup>b</sup>	38.13±1.41 <sup>c</sup>	95.66±0.02 <sup>b</sup>	20.82±0.95 <sup>d</sup>	0.121±0.009
Unripe fruit ethanol	81.17±1.09 <sup>b</sup>	28.83±2.58 <sup>d</sup>	15.17±1.54 <sup>d</sup>	49.82±0.87 <sup>d</sup>	90.51±0.29 <sup>b</sup>	12.57±0.25 <sup>e</sup>	0.184±0.010
Ripe fruit water	24.64±2.14 <sup>d</sup>	90.67±0.24 <sup>b</sup>	41.88±0.99 <sup>c</sup>	21.65±1.26 <sup>f</sup>	92.09±0.04 <sup>b</sup>	7.88±0.09 <sup>f</sup>	0.045±0.001
Ripe fruit ethanol	64.04±1.58 <sup>c</sup>	80.83±0.95 <sup>c</sup>	6.70±0.54 <sup>e</sup>	41.30±0.81 <sup>c</sup>	90.10±0.05 <sup>b</sup>	17.69±0.54 <sup>c</sup>	0.110±0.008
BHA	98.70±0.05 <sup>a</sup>	94.83±0.09 <sup>a</sup>	66.23±0.54 <sup>a</sup>	nt	nt	39.26±0.45 <sup>a</sup>	0.820±0.017
BHT	96.41±0.44 <sup>a</sup>	82.67±0.35 <sup>c</sup>	61.52±0.65 <sup>a</sup>	76.72±0.59 <sup>c</sup>	nt	nt	0.610±0.012
Tocopherol	95.05±0.68 <sup>a</sup>	96.33±0.58 <sup>a</sup>	63.52±0.67 <sup>a</sup>	nt	40.48±0.08 <sup>a</sup>	44.58±0.09 <sup>a</sup>	0.450±0.013
Trolox	nt	nt	nt	90.31±0.09 <sup>a</sup>	nt	nt	nt

**Table 2.** Total phenolic contents of *R. discolor* extracts

Extracts (1 g)	Quercetin Equivalent (mg)	Pyrocatechol Equivalent (mg)
Flower water	93.58±1.25	23.12±0.58
Flower ethanol	152.05±1.36	26.47±0.08
Leaf water	74.54±0.89	20.25±0.25
Leaf ethanol	92.75±1.02	18.72±0.36
Unripe fruit water	69.96±0.12	19.30±0.47
Unripe fruit ethanol	72.06±0.25	18.34±0.29
Ripe fruit water	70.85±0.09	18.82±0.22
Ripe fruit ethanol	91.08±0.22	18.57±0.09

The experimental results were reported as mean ± S.D (standard deviation). Total phenolic compounds as expressed as mg quercetin equivalent /g extract and mg pyrocatechol equivalent / g extract

**Table 3.** Flavonoid contents in *R. discolor* water extracts (µg/g)

Flavonoids	<i>R. discolor</i> leaf water extract	<i>R. discolor</i> flower water extract	<i>R. discolor</i> unripe fruit water extract
Rutin	856	310	336
Apigenin	44	15	8
Myricetin	261	2682	86
Naringin	809	2962	238
Naringenin	41	22	Trace
Quercetin	Trace	5	Trace
Total	2011	5996	668

**Table 4.** The biochemical parameters in serum of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol (mg/mL)	0.411±0.007	<b>0.271±0.014<sup>c</sup></b>	<b>0.324±0.015<sup>c</sup></b>	0.390±0.005
α-tocopherol (mg/mL)	4.03±0.02	<b>2.74±0.12<sup>c</sup></b>	<b>3.54±0.20<sup>a</sup></b>	4.28±0.08
Cholesterol (mg/dL)	50.00±1.80	45.10±1.80	51.00±1.60	<b>63.80±1.60<sup>c</sup></b>
Triglyceride (mg/dL)	66.50±12.20	<b>61.00±6.30<sup>a</sup></b>	69.10±10.40	<b>76.10±7.10<sup>c</sup></b>
HDL (mg/dL)	32.50±2.70	27.80±1.40	30.30±0.80	<b>39.00±2.40<sup>c</sup></b>
LDL (mg/dL)	12.60±0.80	14.80±1.10	<b>16.60±1.30<sup>c</sup></b>	<b>20.60±1.60<sup>c</sup></b>
VLDL (mg/dL)	13.00±2.40	12.10±1.20	13.60±2.10	<b>15.30±1.40<sup>c</sup></b>
GSH-Px (U/mL)	0.250±0.011	0.247±0.016	0.263±0.015	<b>0.320±0.012<sup>c</sup></b>
SOD (U/mL)	6.79±0.24	6.92±0.45	<b>8.39±0.58<sup>a</sup></b>	<b>9.57±0.60<sup>c</sup></b>

Data are mean ± SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:** p<0.05 **b:** p<0.01 **c:** p<0.001

the same group. In the RD treated group compared to the control group, total protein levels were significantly increased in the liver, heart and brain tissues, there is no statistically difference in the spleen tissue, and there were a significantly decrease in the kidney, muscle and lung tissues. In the liver and heart tissues, total protein levels did not differ in the RD plus HP treated group compared to the control group. In the kidney and brain tissues, its level was increased, but it was decreased in the muscle, spleen and lung tissues when compared with the control group (Table 4-11).

#### Cholesterol levels

A significant increase was observed in the cholesterol level of the liver, heart, muscle, brain and lung tissues in the HP treated group compared with the control group. A significant decrease was determined in the kidney and spleen tissues, and also there was any statistically difference between control and HP treated group in the serum. A significant increase in the cholesterol level was observed in the liver, muscle, brain and lung tissues in the RD treated group compared with the control group. While its level was significantly decreased in the kidney and

**Table 5.** The biochemical parameters in liver of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	224.05 $\pm$ 11.35	<b>251.47<math>\pm</math>7.07<sup>a</sup></b>	<b>254.83<math>\pm</math>8.09<sup>a</sup></b>	<b>271.53<math>\pm</math>6.48<sup>c</sup></b>
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	4.24 $\pm$ 0.19	4.39 $\pm$ 0.08	<b>6.45<math>\pm</math>0.10<sup>c</sup></b>	<b>6.57<math>\pm</math>0.12<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	0.120 $\pm$ 0.026	0.116 $\pm$ 0.011	<b>4.251<math>\pm</math>0.131<sup>c</sup></b>	0.088 $\pm$ 0.018
Cholesterol ( $\mu\text{g/g}$ )	950.03 $\pm$ 8.89	<b>1053.93<math>\pm</math>13.85<sup>c</sup></b>	<b>1024.01<math>\pm</math>11.57<sup>c</sup></b>	<b>1191.95<math>\pm</math>9.53<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	1860.63 $\pm$ 38.32	<b>997.84<math>\pm</math>15.06<sup>c</sup></b>	1924.62 $\pm$ 16.87	<b>1479.00<math>\pm</math>17.80<sup>c</sup></b>
Total Protein (mg/g)	81.81 $\pm$ 0.94	<b>65.50<math>\pm</math>2.27<sup>c</sup></b>	<b>86.13<math>\pm</math>1.33<sup>a</sup></b>	84.90 $\pm$ 0.89
MDA (nmol/g)	4.33 $\pm$ 0.09	<b>3.48<math>\pm</math>0.14<sup>c</sup></b>	4.14 $\pm$ 0.15	<b>2.13<math>\pm</math>0.048<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

**Table 6.** The biochemical parameters in kidney of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	2.16 $\pm$ 0.14	2.49 $\pm$ 0.12	2.21 $\pm$ 0.09	<b>1.40<math>\pm</math>0.06<sup>c</sup></b>
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	21.40 $\pm$ 0.14	21.82 $\pm$ 0.24	<b>22.56<math>\pm</math>0.18<sup>b</sup></b>	<b>17.91<math>\pm</math>0.21<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	3.184 $\pm$ 0.190	<b>0.864<math>\pm</math>0.077<sup>c</sup></b>	<b>0.305<math>\pm</math>0.053<sup>c</sup></b>	2.842 $\pm$ 0.154
Cholesterol ( $\mu\text{g/g}$ )	1682.51 $\pm$ 18.86	<b>1575.19<math>\pm</math>12.43<sup>c</sup></b>	<b>1606.21<math>\pm</math>14.11<sup>b</sup></b>	1636.20 $\pm$ 17.83
GSH ( $\mu\text{g/g}$ )	331.40 $\pm$ 12.53	<b>131.14<math>\pm</math>1.51<sup>c</sup></b>	<b>393.31<math>\pm</math>15.79<sup>b</sup></b>	<b>733.10<math>\pm</math>16.15<sup>c</sup></b>
Total Protein (mg/g)	51.52 $\pm$ 0.93	<b>41.86<math>\pm</math>0.42<sup>c</sup></b>	<b>42.68<math>\pm</math>0.24<sup>c</sup></b>	<b>61.27<math>\pm</math>0.31<sup>c</sup></b>
MDA (nmol/g)	4.13 $\pm$ 0.18	<b>5.18<math>\pm</math>0.18<sup>c</sup></b>	3.88 $\pm$ 0.18	<b>0.55<math>\pm</math>0.04<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

**Table 7.** The biochemical parameters in heart of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	0.167 $\pm$ 0.012	<b>0.219<math>\pm</math>0.018<sup>a</sup></b>	0.167 $\pm$ 0.013	0.170 $\pm$ 0.015
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	5.66 $\pm$ 0.24	6.02 $\pm$ 0.16	<b>5.13<math>\pm</math>0.13<sup>a</sup></b>	<b>3.88<math>\pm</math>0.05<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	2.458 $\pm$ 0.110	<b>0.076<math>\pm</math>0.004<sup>c</sup></b>	<b>0.103<math>\pm</math>0.018<sup>c</sup></b>	<b>0.579<math>\pm</math>0.100<sup>c</sup></b>
Cholesterol ( $\mu\text{g/g}$ )	801.58 $\pm$ 3.16	<b>906.56<math>\pm</math>1.09<sup>c</sup></b>	790.12 $\pm$ 3.95	<b>660.08<math>\pm</math>6.03<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	23.16 $\pm$ 0.41	<b>21.73<math>\pm</math>0.17<sup>a</sup></b>	<b>25.21<math>\pm</math>0.61<sup>b</sup></b>	<b>28.42<math>\pm</math>0.15<sup>c</sup></b>
Total Protein (mg/g)	32.55 $\pm$ 0.47	<b>31.37<math>\pm</math>0.24<sup>a</sup></b>	<b>38.89<math>\pm</math>0.26<sup>c</sup></b>	32.07 $\pm$ 0.17

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

**Table 8.** The biochemical parameters in muscle of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	0.153 $\pm$ 0.063	0.099 $\pm$ 0.008	0.188 $\pm$ 0.014	0.117 $\pm$ 0.005
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	2.48 $\pm$ 0.09	2.82 $\pm$ 0.13	<b>5.83<math>\pm</math>0.05<sup>c</sup></b>	<b>14.96<math>\pm</math>0.13<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	2.32 $\pm$ 0.15	<b>1.89<math>\pm</math>0.12<sup>a</sup></b>	2.35 $\pm$ 0.08	2.06 $\pm$ 0.12
Cholesterol ( $\mu\text{g/g}$ )	335.57 $\pm$ 7.77	<b>439.16<math>\pm</math>9.47<sup>c</sup></b>	<b>470.16<math>\pm</math>3.93<sup>c</sup></b>	<b>432.67<math>\pm</math>4.18<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	138.55 $\pm$ 1.32	<b>118.06<math>\pm</math>1.43<sup>c</sup></b>	138.87 $\pm$ 1.25	<b>117.16<math>\pm</math>1.11<sup>c</sup></b>
Total Protein (mg/g)	45.20 $\pm$ 0.56	<b>32.51<math>\pm</math>0.78<sup>c</sup></b>	<b>32.63<math>\pm</math>0.70<sup>c</sup></b>	<b>34.15<math>\pm</math>0.40<sup>c</sup></b>
MDA (nmol/g)	26.08 $\pm$ 0.28	25.78 $\pm$ 0.39	<b>14.50<math>\pm</math>0.11<sup>c</sup></b>	<b>30.34<math>\pm</math>0.19<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

spleen tissues, there was not any statistical difference between control and RD treated group in the serum and heart tissues. A significant increase was observed in the cholesterol level of the liver, muscle, serum, brain and lung tissues in the RD plus HP treated group compared with the control group. A significant decrease was determined in the heart and spleen tissues, and any difference was not observed between control

and RD plus HP treated group in the kidney tissue (Table 4-11).

#### *Vitamin A (Retinol) levels*

In comparison to the control group, there were an increase in the retinol level in the liver and heart tissues in the HP treated group at the end of the 10<sup>th</sup> week. However, the retinol level was decreased in the serum and lung tissues, but its level did not show any difference in the kidney, muscle and brain

**Table 9.** The biochemical parameters in brain of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	0.044 $\pm$ 0.006	0.038 $\pm$ 0.001	<b>0.032<math>\pm</math>0.002<sup>a</sup></b>	0.039 $\pm$ 0.001
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	9.11 $\pm$ 0.19	9.69 $\pm$ 0.30	9.59 $\pm$ 0.17	8.71 $\pm$ 0.07
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	3.31 $\pm$ 0.14	<b>7.59<math>\pm</math>0.15<sup>c</sup></b>	<b>7.99<math>\pm</math>0.15<sup>c</sup></b>	<b>6.40<math>\pm</math>0.12<sup>c</sup></b>
Cholesterol ( $\mu\text{g/g}$ )	1511.37 $\pm$ 17.00	<b>1641.87<math>\pm</math>12.75<sup>c</sup></b>	<b>1638.71<math>\pm</math>9.24<sup>c</sup></b>	<b>1658.58<math>\pm</math>7.24<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	33.97 $\pm$ 0.25	<b>28.60<math>\pm</math>0.25<sup>c</sup></b>	<b>34.81<math>\pm</math>0.20<sup>b</sup></b>	<b>25.41<math>\pm</math>0.07<sup>c</sup></b>
Total Protein (mg/g)	16.36 $\pm$ 0.16	15.94 $\pm$ 0.14	<b>17.60<math>\pm</math>0.08<sup>c</sup></b>	<b>16.90<math>\pm</math>0.17<sup>a</sup></b>
MDA (nmol/g)	2.860 $\pm$ 0.159	<b>1.246<math>\pm</math>0.154<sup>c</sup></b>	<b>0.397<math>\pm</math>0.094<sup>c</sup></b>	<b>0.118<math>\pm</math>0.031<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

**Table 10.** The biochemical parameters in spleen of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	1.96 $\pm$ 0.11	<b>1.37<math>\pm</math>0.07<sup>c</sup></b>	<b>1.27<math>\pm</math>0.06<sup>c</sup></b>	<b>0.85<math>\pm</math>0.11<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	6.93 $\pm$ 0.19	<b>4.13<math>\pm</math>0.04<sup>c</sup></b>	<b>4.04<math>\pm</math>0.18<sup>c</sup></b>	<b>2.93<math>\pm</math>0.15<sup>c</sup></b>
Cholesterol ( $\mu\text{g/g}$ )	1961.36 $\pm$ 11.97	<b>1691.99<math>\pm</math>20.91<sup>c</sup></b>	<b>1840.63<math>\pm</math>11.66<sup>c</sup></b>	<b>1063.06<math>\pm</math>11.94<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	48.40 $\pm$ 0.82	<b>50.69<math>\pm</math>0.45<sup>a</sup></b>	<b>41.28<math>\pm</math>0.57<sup>c</sup></b>	<b>32.25<math>\pm</math>0.79<sup>c</sup></b>
Total Protein (mg/g)	60.51 $\pm$ 0.40	<b>54.29<math>\pm</math>1.48<sup>c</sup></b>	58.35 $\pm$ 0.51	<b>53.31<math>\pm</math>0.59<sup>c</sup></b>
MDA (nmol/g)	68.28 $\pm$ 0.45	<b>95.86<math>\pm</math>0.77<sup>c</sup></b>	65.14 $\pm$ 0.93	<b>91.58<math>\pm</math>1.74<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

tissues in the HP treated group compared with the control group. When the RD treated group was compared with the control group, there was a significant increase in the retinol level in the liver tissue. The retinol level was decreased in the serum and brain tissues, but its level was not statistically different in the kidney, heart, muscle and lung tissues when compared with the control group. Compared to the control group, the retinol level in the liver tissue in the RD plus HP treated group was significantly increased. However, the

retinol level was decreased in the kidney and lung tissues, while its level was not different in the serum, heart, muscle and brain tissues compared to the control group (Table 4-11).

#### *Vitamin E ( $\alpha$ -tocopherol and $\alpha$ -tocopherol acetate) levels*

The  $\alpha$ -tocopherol level was significantly decreased in the serum and spleen tissues in the HP treated group compared to the control group. A significant increase in the  $\alpha$ -tocopherol level was observed in the brain and

lung tissues, while its level did not show any statistically difference in the liver, kidney, heart and muscle tissues in the HP treated group compared with the control group. The  $\alpha$ -tocopherol level was significantly increased in the liver, kidney, muscle and brain tissues in the RD treated group in comparison to the control group. However, its level was significantly decreased in the serum, heart and spleen tissues, but it is not statistically different between control and the RD treated group in the lung tissue. A significant increase in the  $\alpha$ -tocopherol level was observed in the liver, muscle and brain tissues in the RD plus HP treated group in comparison to the control group. The  $\alpha$ -tocopherol level was decreased in the kidney, heart, spleen and lung tissues,

significantly increase in the  $\alpha$ -tocopherol acetate level in the liver and brain tissues. On the other hand, the  $\alpha$ -tocopherol acetate level was decreased in the kidney, heart, spleen and lung tissues, but its level was not statistically different in the muscle tissue in the RD treated group compared to the control group. Compared with the control group, there was a significantly increase in the  $\alpha$ -tocopherol acetate level in the brain and lung tissues in the RD plus HP treated group. The  $\alpha$ -tocopherol acetate level was decreased in the heart and spleen tissues, but its level did not show any statistically difference in the liver, kidney and muscle tissues in the RD plus HP treated group in comparison to the control group (Table 4-11).

**Table 11.** The biochemical parameters in lung of Wistar rats

Biochemical Parameters	Control	HP	RD	RD+HP
Retinol ( $\mu\text{g/g}$ )	4.66 $\pm$ 0.12	<b>3.14<math>\pm</math>0.11<sup>c</sup></b>	4.42 $\pm$ 0.10	<b>3.81<math>\pm</math>0.17<sup>c</sup></b>
$\alpha$ -tocopherol ( $\mu\text{g/g}$ )	4.34 $\pm$ 0.15	<b>5.47<math>\pm</math>0.15<sup>c</sup></b>	4.77 $\pm$ 0.17	<b>3.08<math>\pm</math>0.17<sup>c</sup></b>
$\alpha$ -tocopherol acetate ( $\mu\text{g/g}$ )	5.94 $\pm$ 0.18	5.46 $\pm$ 0.10	<b>3.25<math>\pm</math>0.18<sup>c</sup></b>	<b>7.50<math>\pm</math>0.14<sup>c</sup></b>
Cholesterol ( $\mu\text{g/g}$ )	1122.10 $\pm$ 11.58	<b>1281.48<math>\pm</math>14.26<sup>c</sup></b>	<b>1390.07<math>\pm</math>28.42<sup>c</sup></b>	<b>1300.38<math>\pm</math>11.56<sup>c</sup></b>
GSH ( $\mu\text{g/g}$ )	14.79 $\pm$ 0.28	<b>10.92<math>\pm</math>0.29<sup>c</sup></b>	<b>13.56<math>\pm</math>0.19<sup>c</sup></b>	<b>13.13<math>\pm</math>0.18<sup>c</sup></b>
Total Protein (mg/g)	30.00 $\pm$ 0.44	<b>23.04<math>\pm</math>0.42<sup>c</sup></b>	<b>25.86<math>\pm</math>0.61<sup>c</sup></b>	<b>25.93<math>\pm</math>0.43<sup>c</sup></b>
MDA (nmol/g)	43.58 $\pm$ 0.50	<b>45.90<math>\pm</math>0.59<sup>b</sup></b>	<b>39.76<math>\pm</math>0.62<sup>c</sup></b>	<b>38.31<math>\pm</math>0.09<sup>c</sup></b>

Data are mean  $\pm$  SD values for ten rats in each group. There is not statistically difference among in the same letter groups. All the groups were compared with control group. **a:**  $p < 0.05$  **b:**  $p < 0.01$  **c:**  $p < 0.001$

and there was no statistically difference between control and RD plus HP treated group in the serum (Table 4-11).

In comparison to the control group, there was a decrease in the  $\alpha$ -tocopherol acetate level in the kidney, heart, muscle and spleen tissues in the HP treated group. Nevertheless, the  $\alpha$ -tocopherol acetate level was increased in the brain tissue, but its level was not statistically different in the liver and lung tissues in the HP treated group compared with the control group. When the RD treated group was compared with the controls, there was a

## DISCUSSION

Different antioxidant compounds may act through different mechanisms; consequently, one method alone can not be utilized to fully evaluate the antioxidant capacity of herbal extracts and does not reflect *in vitro* antioxidant capacity of pure compounds. For this reason, different *in vitro* antioxidant tests were carried out using different approaches and mechanisms.

#### *ABTS<sup>•+</sup> radical scavenging capacity*

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and biological systems. The improved technique for the generation of ABTS<sup>•+</sup> described here involves the direct production of the blue/green ABTS<sup>•+</sup> chromophore through reaction between ABTS and potassium persulfate (41). In our study, RD extracts showed scavenging activity on ABTS radical in the range of 24.64-97.30%. ABTS scavenging activities of RD leaf ethanol, leaf water and flower ethanol are higher than BHT and tocopherol.

#### *Superoxide anion scavenging capacity*

In this method, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces the yellow dye (NBT<sup>2+</sup>) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (42,43). The decrease in the absorbance value at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. In the present study, the scavenging activity of superoxide anion for RD extracts was found to in the range of 28.83-90.67%. Ripe fruit water, flower water and leaf water extracts showed higher superoxide scavenging activity.

#### *Ferrous ion chelating activity*

Among the transition metals, iron is known as the most important pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. Fe<sup>3+</sup> ion also produces radicals from peroxides, although the rate is ten fold less than that of Fe<sup>2+</sup> ion (44). Fe<sup>2+</sup> ion is the most powerful pro-oxidant among various species of metal ions (45). Chelation of the ferrous ions by *R. discolor* extracts was estimated by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe<sup>2+</sup>. The complex formation is inhibited and the red colour of the complex fades in the presence of chelating agents. By measuring the colour reduction, therefore, it is possible to estimate the chelating activity of the co-existing chelator (46). In this assay, the

natural compound interfered with the formation of the ferrozine-Fe<sup>2+</sup> complex, suggesting that it has chelating activity and captures ferrous ions before ferrozine. In this study, ferrous ion chelating of RD extracts were in the range of 6.70-54.58%. All extracts showed lower activity of metal chelating than BHA, BHT and tocopherol.

#### *DPPH<sup>•</sup> radical scavenging capacity*

The effect of antioxidants on DPPH<sup>•</sup> radical scavenging was presumed to be due to their hydrogen donating ability. DPPH<sup>•</sup> is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH<sup>•</sup> radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discolouration from purple to yellow (41). In our study, the scavenging activities of DPPH radical for RD extracts were found to in the range of 21.65-84.42%. Flower water, flower ethanol, leaf water and leaf ethanol extracts showed higher scavenging activity than BHT.

#### *Hydrogen peroxide scavenging and reducing power capacity*

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic for the cells because it may give rise to hydroxyl radical in the cells (14). Thus, the removing of H<sub>2</sub>O<sub>2</sub> is very important for antioxidant defense in living organisms. In our study, the results for RD extracts in H<sub>2</sub>O<sub>2</sub> scavenging and reducing power assays were in the range of 7.88-98.77% and 0.110-1.589 (absorbance), respectively. Flower ethanol extract showed higher activity than BHA and tocopherol for these assays.

#### *Inhibition on lipid peroxidation*

Antioxidant activity is defined as the ability of a compound to inhibit oxidative degradation, such as lipid peroxidation (47). The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. In the present study, RD extracts were in the range of 90.10-95.66% for inhibition of lipid peroxidation.

Activities of all extracts were higher than tocopherol for inhibition of lipid peroxidation.

#### *Total phenolic compounds*

Phenols are very important plant constituents because of the radical scavenging ability of their hydroxyl groups (48). In the present study, the highest phenolic compounds in the RD flower ethanol and flower water extracts were 152.05 mg/g, 93.58 mg/g quercetin, 26.47 mg/g, 23.12 mg/g pyrocatechol, respectively.

#### *Total flavonoid contents*

Flavonoids are plant phenolic compounds with strong antioxidant properties found in many dietary sources such as tea, onion, broccoli, apple and green beans (49). Flavonoids can prevent oxidative damage as a result of their ability to scavenge against reactive oxygen species such as hydroxyl radical and superoxide anion (50) and metal chelating (51). In our study, highest total flavonoid contents were observed in the RD flower extract. The flower extracts showed antioxidant activity at least up to the standard antioxidants or higher than these standards in all *in vitro* tests.

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 enzymes (52). MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids, and thus increased MDA content which is an important indicator of lipid peroxidation (53). Previous studies have shown that treating of *Rubus* sp. extracts were decreased and/or prevented MDA levels in various tissues (54-58). In our study, MDA levels were decreased or prevented in the RD treated and RD plus HP treated groups in the liver, kidney, muscle, brain, spleen and lung tissues. So, this might be due to inhibitory effect of *R. discolor* on lipid peroxidation.

SOD is the most important defense mechanism against toxic effects of ROS. It accelerates the dismutation of  $H_2O_2$ , also termed as a primary defence, as it prevents further generation of free radicals. Catalase (CAT) helps in the removal of  $H_2O_2$  formed during the reaction catalyzed by SOD (59). It

has been reported that the increase of SOD activity can be known to serve as protective responses to eliminate reactive free radicals (60). In some studies, it has been indicated that superoxide radicals can inhibit CAT activity and the increase of  $H_2O_2$  resulting from CAT inhibition could finally inhibit SOD activity (61). In this study, SOD activity was increased significantly in the serum of RD treated and RD plus HP treated groups. Similarly, in the previous studies, it has been reported that SOD activity was increased in various tissues by *Rubus* sp. extracts treated different organisms, such as mice, hamster, human, rat (55,57,58,62,63). The increase of SOD activity might be associated with increasing effect of RD extracts on rat serum, because, it was observed that SOD activity increased in the RD treated and RD plus HP treated groups.

Glutathione peroxidase (GSH-Px) is an antioxidant selenoenzyme and is present in the cytosol of cells or plasma. The kidney secretes the GSH-Px into plasma. The major function of this enzyme, used in the glutathione as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases (53,64). GSH-Px converts hydrogen peroxide into  $H_2O$  in the presence of oxidized glutathione (GSSG) (65). It has been reported that *Rubus* sp. extracts were significantly effected GSH-Px activity in various organism tissues (62,63,66). In the present study, the increase of GSH-Px activity might reflect cellular oxidative stress due to  $H_2O_2$  exposure or increasing effect of RD extracts in the RD plus HP treated in the rat serum. Kumar et al. (67) have been reported that GSH-Px activity was significantly increased in the rat liver tissues due to  $H_2O_2$  treatment.

High levels of free radicals or active oxygen species create oxidative stress which leads to a variety of biochemical and physiological lesions often resulting in metabolic impairment and cell death. SOD catalyzes the breakdown of  $O_2^-$  to  $O_2$  and  $H_2O_2$ , removes singlet oxygen as well as  $O_2^-$ , prevents formation of  $OH^-$  (68), and has been implicated as an essential defense against the potential toxicity of oxygen (69). GSH-Px may be responsible for scavenging  $H_2O_2$ , catalyzing the peroxidation of reduced glutathione (GSH), and forming the oxidized

disulfide form of glutathione (GSSG) as a product. Glutathione is the major low molecular weighted thiol compound in most plants and exists in both a reduced form (GSH) and an oxidized form, glutathione disulfide (GSSG). The reduced form of glutathione plays an important role in the stabilization of many enzymes. It also reacts directly with free radicals including hydroxyl radical to prevent the inactivation of enzymes by oxidation of the essential thiol group. The majority of glutathione in the cell is maintained in the reduced state (70). In this study, GSH levels were increased significantly in the liver, kidney, heart and brain tissues of RD treated and RD plus HP treated groups. Similarly, in the previous study, GSH levels were increased in the rat serum by treating of *Rubus* sp. extracts (66). The increase in the GSH levels might be associated with increasing effect on the antioxidant potential of RD extracts on rat serum. However, its level was significantly decreased in the liver, kidney, heart, muscle, brain and lung tissues of HP treated group. This increase can be attributed to toxicity and oxidized effects of H<sub>2</sub>O<sub>2</sub>. Kumar et al. (67) determined that GSH level was decreased in the rat liver tissue due to H<sub>2</sub>O<sub>2</sub> treatment. GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes such as GSH-Px. Under oxidative stress, GSH is consumed by the GSH reductase to detoxify peroxides produced due to increase of lipid peroxidation.

In this study, total protein levels were significantly decreased in the liver, kidney, heart, muscle, spleen and lung tissues of HP treated groups. The decrease of total protein levels might be associated with toxicity and oxidized effects of H<sub>2</sub>O<sub>2</sub> on the living organism.

In the present study, we have observed that the triglycerides, HDL, LDL, VLDL and cholesterol levels were increased in the RD plus HP treatment group without influencing in the HP treated and RD treated groups in the rat serum. In addition, cholesterol levels were decreased in the kidney, heart and spleen tissues of RD treated groups. However, its level was significantly increased in the liver, muscle, brain and lung tissues of RD treated

groups. In the previous studies, *Rubus* sp. extracts have decreased cholesterol levels in the rat and hamsters (56,71).

Vitamin E is a common term used for tocopherols and tocotrienols. It is an important antioxidant that is directly involved in scavenging oxygen free radicals and quenching lipid peroxidation chain reactions that occur during oxidation reactions with poly unsaturated fatty acids (PUFA) (72). Vitamin E reactions result in the formation of tocopheroxyl radicals that react with other antioxidants to regenerate the active molecule (73,74). Ghalayini et al. (57) showed that treating of *R. idaeus* water extract was increased the vitamin E level in kidney tissue of mice. In our study, vitamin E level increased in the RD treated groups in the liver, kidney and muscle tissues. It can be said that treatment of RD flowers extract increased vitamin E levels in these tissues.

Vitamin A plays a vital role in the development and homeostasis of almost every vertebrate tissue by regulating embryogenesis, cell differentiation, proliferation, metabolism, and apoptosis (75,76). Vitamin A (retinol) is obtained in the diet in the form of retinyl esters or through the ingestion of  $\beta$ -carotene, which is converted to two molecules of retinol. The carboxylic acid form of vitamin A (all-*trans*-retinoic acid) has important effects on the development of the cardiovascular system (76). Epidemiologic evidences have suggested that vitamin A is an important dietary factor for decreasing the incidence of heart disease (77,78). In our study, while vitamin A level was decreased in the RD treated group in the serum, brain and spleen tissues, its level was increased in the RD treated group in the liver tissue. It can be speculated that treatment of RD flowers extract affected vitamin A levels in these tissues. Keser et al. (79) have indicated the *R. discolor* flower water extract significantly affect the vitamin D and K levels against hydrogen peroxide administered Wistar rat tissues.

In previous studies, it has been determined that antioxidant properties of other species in the genus *Rubus* as *in vivo* and/or *in vitro*. However, we did not find any study about antioxidant properties of *R. discolor* extracts in literatures. As a result of this study for the

first time the antioxidant properties of *R. discolor* water and ethanol extracts were determined *in vitro* the antioxidant effects of RD flower water extract in Wistar rats were investigated. In tissues of hydrogen peroxide treated groups, while MDA level was increased, GSH and total protein levels were decreased; in RD flower extract given group, these substances levels were protected. In hydrogen peroxide and RD flower extract treated groups, SOD and GSH-Px levels were increased. In addition, it was observed that vitamin A and E, and cholesterol levels were affected by the administration of hydrogen peroxide and RD flower extract. The present study results showed the *R. discolor* extract have the protective effects against H<sub>2</sub>O<sub>2</sub> via its free radical scavenging activity and it affected levels of antioxidant enzymes, lipophylic vitamins, cholesterol, GSH, total protein and MDA.

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## REFERENCES

- Patel AV, Rojas-Vera J, Dacke CG, Therapeutic constituents and actions of *Rubus* species, *Curr Med Chem* 11, 1501–1512, 2004.
- Blumenthal M, The complete commission German E monographs: Therapeutic guide to herbal medicines, 1st Ed, American Botanical Council, Texas, USA, 1998.
- Zhang Y, Zhang Z, Yang Y, Zu X, Guan D, Wang Y, Diuretic activity of *Rubus idaeus* L (Rosaceae) in rats, *Trop J Pharm Res* 10, 243–248, 2011.
- Tanaka T, Tachibana H, Nonaka G, Nishioka I, Hsu FL, Kohda H, Tanaka O, Tannins and related compounds 122. New dimeric, trimeric and tetrameric ellagitannins, Lambertianins A–D, from *Rubus lambertianus* Seringe, *Chem Pharma Bull* 41, 1214–1220, 1993.
- Vrhovsek U, Palchetti A, Reniero F, Guillou C, Masuero D, Mattivi F, Concentration and mean degree of polymerization of *Rubus* ellagitannins evaluated by optimized acid methanolysis, *J Agr Food Chem* 54, 4469–4475, 2006.
- Hussein SAM, Ayoub NA, Nawwar MAM, Caffeoyl sugar esters and an ellagitannin from *Rubus sanctus*, *Phytochemistry* 63, 905–911, 2003.
- Badr AM, El-Demerdash E, Khalifa AE, Ghoneim AI, Ayoub NA, Abdel-Naim AB, *Rubus sanctus* protects against carbon tetrachloride-induced toxicity in rat isolated hepatocytes: isolation and characterization of its galloylated flavonoids, *J Pharm Pharmacol* 61, 1511–1520, 2009.
- Sun Y, Free radicals, antioxidant enzymes and carcinogenesis, *Free Radical Bio Med* 8, 583–599, 1990.
- Gulcin I, Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), *Toxicology* 217, 213–220, 2006.
- Gulcin I, Bursal E, Sehitoglu MH, Bilsel M, Goren AC, Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey, *Food Chem Toxicol* 48, 2227–2238, 2010.
- Halliwell B, Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans, *Free Radical Res* 25, 57–74, 1996.
- Gulcin I, Mshvildadze V, Gepdiremen A, Elias R, Screening of antiradical and antioxidant activity of monodesmosides and crude extract from *Leontice smirnowii* tuber, *Phytomedicine* 13, 343–351, 2006.
- Vendemiale G, Grattagliano I, Altomare E, An update on the role of free radicals and antioxidant defense in human disease, *Int J Clin Lab Res* 29, 49–55, 1999.
- Halliwell B, Reactive oxygen species in living systems-source, biochemistry, and role in human-disease, *Am J Med* 91, 14–22, 1991.
- Kaur H, Perkins J, The free radical chemistry of food additives. In, Aruoma OI Halliwell B (Ed): *Free radicals and food additives*, 17–35, Taylor and Francis Ltd, London, 1991.
- Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT, Hydrogen peroxide and nitric oxide as signalling molecules in plants, *J Exp Bot* 53, 1237–1247, 2002.
- Rhee SG, Bae YS, Lee SR, Kwon J, Hydrogen peroxide: A key messenger that modulates protein phosphorylation through cysteine oxidation, *Science's STKE*: [http://www.stke.org/cgi/content/full/OC\\_sigtrans;2000/53/pe1](http://www.stke.org/cgi/content/full/OC_sigtrans;2000/53/pe1), 2000.
- Finkel T, Redox-dependent signal transduction, *FEBS Lett* 476, 52–54, 2000.
- Neill S, Desikan R, Hancock J, Hydrogen peroxide signaling, *Curr Opin Plant Biol* 5, 388–395, 2002.

20. Keser S, Celik S, Turkoglu S, Total phenolic contents and free-radical scavenging activities of grape (*Vitis vinifera* L.) and grape products, *Int J Food Sci Nutr* 64, 210-216, 2013.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Bio Med* 26, 1231-1237, 1999.
22. Shimada K, Fujikawa K, Yahara K, Nakamura T, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J Agr Food Chem* 40, 945-948, 1992.
23. Liu F, Ooi VEC, Chang ST, Free radical scavenging activities of mushroom polysaccharide extracts, *Life Sci* 60, 763-771, 1997.
24. Dinis TCP, Madeira VMC, Almeida LM, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid-peroxidation and as peroxyl radical scavengers, *Arch Biochem Biophys* 315, 161-169, 1994.
25. Oyaizu M, Studies on product of browning reaction prepared from glucose amine, *Jpn J Nutr* 44, 307-315, 1986.
26. Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, *Carcinogenesis* 10, 1003-1008, 1989.
27. Mitsuda H, Yuasumoto K, Iwami K, Antioxidation action of indole compounds during the autoxidation of linoleic acid, *Eiyo to Shokuryo* 19, 210-214, 1996.
28. Slinkard K, Singleton VL, Total phenol analysis-automation and comparison with manual methods, *Am J Enol Viticult* 28, 49-55, 1977.
29. Zu YG, Li CY, Fu YJ, Zhao CJ, Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaf by RP-HPLC with DAD, *J Pharmaceut Biomed* 41, 714-719, 2006.
30. Keser S, Yilmaz Ö, Tuzcu M, Effects of resveratrol on fatty acid levels in serum and erythrocytes of rats administered potassium bromate, *Asian J Chem* 22, 7841-7849, 2010.
31. Keser S, Determination of total antioxidant activities of yarrow (*Achillea millefolium*), hawthorn (*Crataegus monogyna*) and blackberry (*Rubus discolor*) and investigation of their effects on some biochemical parameters in oxidative stress generated rats, Ph. D. Thesis, Firat University, Sciences Institute, Chemistry Department. Elazig/TURKEY, (In Turkish), 2012.
32. Jain SK, McVie R, Duett J, Herbst JJ, Erythrocyte membrane lipid peroxidation and glycolylated hemoglobin in diabetes, *Diabetes* 38, 1539-1543, 1989.
33. Beutler E, Dubon OB, Kelly M, Improved method for the determination of blood glutathione, *J Lab Clin Med* 61, 882-888, 1963.
34. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin-phenol reagent, *J Biochem* 193, 265-277, 1951.
35. Paglia DE, Valentine WN, Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J Lab Clin Med* 70, 158-169, 1967.
36. McCord JM, Fridovich I, Superoxide dismutase, an enzymatic function for erythrocyte hemocuprein (hemocuprein), *J Bio Chem* 244, 6049-6053, 1969.
37. Yilmaz O, Keser S, Tuzcu M, Cetintas B, Resveratrol (trans-3,4',5-trihydroxystilbene) decreases lipid peroxidation level and protects antioxidant capacity in sera and erythrocytes of old female Wistar rats induced by the kidney carcinogen potassium bromate, *ETAP* 24, 79-85, 2007.
38. Keser S, Yilmaz O, Tuzcu M, Erman O, Irtegun S, The effects of catechin, lipoic acid, resveratrol and potassium bromate on fatty acid, lipophylic vitamins and cholesterol levels in muscle of Wistar rats, *J Chem Soc Pakistan* 34, 89-93, 2012.
39. Bragagnolo N, Rodriguez-Amaya DB, Comparison of the cholesterol content of Brazilian chicken and quail eggs, *J Food Comp Anal* 16, 147-153, 2003.
40. Katsanidis E, Addis PB, Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue, *Free Radical Bio Med* 27, 1137-1140, 1999.
41. Keser S, Turkoglu S, Celik S, Turkoglu I, Determination of antioxidant capacities of *Phlomis pungens* Willd. var. *hispida* Hub.-Mor, *Asian J Chem* 24, 2780-2784, 2012.
42. Cos P, Ying LY, Calomme M, Hu JH, Cimanga K, Van Poel B, Pieters L, Vlietinck AJ, Berghe DV, Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers, *J Nat Prod* 61, 71-76, 1998.
43. Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J, Codina C, Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and

- aromatic plants, *J Agr Food Chem* 50, 6882–6890, 2002.
44. Miller DD, Mineral, Food Chemistry, in ed.: O.R. Fennema, Marcel Dekker, New York, USA, 1996.
  45. Halliwell B, Gutteridge JMC, Oxygen toxicity, oxygen radicals, transition metals and disease, *Biochem J* 219, 1–14, 1984.
  46. [46] Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H, Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind, *J Agr Food Chem* 48, 180–185, 2000.
  47. Roginsky V, Lissi EA, Review of methods to determine chain-breaking antioxidant activity in food, *Food Chem* 92, 235–254, 2005.
  48. Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E, Effects of the interaction of tannins with co-existing substances.6. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical, *Chem Pharm Bull* 37, 2016–2021, 1989.
  49. Anjaneyulu M, Chopra K, Quercetin, an antioxidant bioflavonoid, attenuates diabetic nephropathy in rats, *Clin Exp Pharmacol P* 31, 244–248, 2004.
  50. Galati G, Sabzevari O, Wilson JX, O'Brien PJ, Prooxidant activity and cellular effects of phenoxy radicals of dietary flavonoids and other polyphenolics, *Toxicology* 177, 91–104, 2002.
  51. Pedrielli P, Skibsted LH, Antioxidant synergy and regeneration effect of quercetin, (–)-epicatechin, and (+)-catechin on  $\alpha$ -tocopherol in homogeneous solutions of peroxidating methyl linoleate, *J Agr Food Chem* 50, 7138–7144, 2002.
  52. Goel A, Dani V, Dhawan DK, Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity, *Chem Biol Interact* 156, 131–140, 2005.
  53. Demir F, Uzun FG, Durak D, Kalender Y, Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin, *Pestic Biochem Phys* 99, 77–81, 2011.
  54. Broomfield AM, Hendriks WH, Hunt MB, McGhie TK, The *in vivo* antioxidant action and the reduction of oxidative stress by boysenberry extract is dependent on base diet constituents in rats, *J Med Food* 10, 281–289, 2007.
  55. Hong Z, Chen W, Zhao J, Wu Z, Zhou JH, Li T, Hu J, Hepatoprotective effects of *Rubus alaeifolius* Poir. and identification of its active constituents, *J Ethnopharmacol* 129, 267–272, 2010.
  56. De Araujo PRF, Santos VD, Machado AR, Fernandes CG, Silva JA, Rodrigues RD, Benefits of blackberry nectar (*Rubus* spp.) relative to hypercholesterolemia and lipid peroxidation, *Nutricion Hospitalaria* 26, 984–990, 2011.
  57. Ghalayini IF, Al-Ghazo MA, Harfeil MNA, Prophylaxis and therapeutic effects of raspberry (*Rubus idaeus*) on renal stone formation in Balb/c mice, *Int Braz J Urol* 37, 259–266, 2011.
  58. Gao J, Sun CR, Yang JH, Shi JM, Du YG, Zhang YY, Li JH, Wan HT, Evaluation of hepatoprotective and antioxidant activities of *Rubus parvifolius* L. *J Zhejiang Univ Sci B* 12, 135–142, 2011.
  59. Liu CM, Zheng YL, Lu J, Zhang ZF, Fan SH, Wu DM, Ma JQ, Quercetin protects rat liver against lead-induced oxidative stress and apoptosis, *ETAP* 29, 158–166, 2010.
  60. Celik I, Suzek H, Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats, *Ecotox Environ Safe* 72, 905–908, 2009.
  61. Gultekin F, Ozturk M, Akdogan M, The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro), *Arch Toxicol* 74, 533–538, 2000.
  62. Jiao H, Wang SY, Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry, *J Agr Food Chem* 48, 5672–5676, 2000.
  63. Wang SY, Jiao H, Changes in oxygen-scavenging systems and membrane lipid peroxidation during maturation and ripening in blackberry, *J Agr Food Chem* 49, 1612–1619, 2001.
  64. Bebe FN, Panemangalore M, Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats, *J Environ Sci Heal B* 38, 349–363, 2003.
  65. Kanbur M, Eraslan G, Silici S, Antioxidant effect of propolis against exposure to propetamphos in rats, *Ecotox Environ. Safe* 72, 909–915, 2009.
  66. Lee YI, Whang KE, Cho JS, Ahn BM, Lee SB, Dong MS, Kim TH, *Rubus coreanus* extract attenuates acetaminophen induced hepatotoxicity; involvement of cytochrome P450 3A4, *Biomolecules Therapeutics* 17, 455–460, 2009.
  67. Kumar S, Srivastava N, Gomes J, The effect of lovastatin on oxidative stress and antioxidant

- enzymes in hydrogen peroxide intoxicated rats, Food Chem Toxicol 49, 898–902, 2011.
68. Fridovich I, Superoxide radical and superoxide dismutase, Biochem Soc T 1, 48–50, 1973.
  69. McCord JM, Superoxide dismutases: occurrence, structure, function and evolution, In Isozyme: Current Topics in Biological and Medical Research; Rattazzi, M., Scandalios, J., Whitt, G.S., Eds.; Liss: New York, Vol. 3, pp 1-21, 1979.
  70. Kosower NS, Kosower EM, The glutathione status of cells, Int Rev Cytology 54, 109–160, 1978.
  71. Koh GY, McCutcheon K, Zhang F, Liu D, Cartwright CA, Martin R, Yang P, Liu Z, Improvement of obesity phenotype by Chinese sweet leaf tea (*Rubus suavissimus*) components in high-fat diet-induced obese rats, J Agr Food Chem 59, 98–104, 2011.
  72. Sattler SE, Gilliland LU, Magallanes-Lundback M, Pollard M, DellaPenna D, Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination, Plant Cell 16, 1419–1432, 2004.
  73. Carelli AA, Franco CI, Crapiste HG, Effectiveness of added natural antioxidants in sunflower oil, Grasas y Aceites 56, 303–310, 2005.
  74. Liebler DC, The role of metabolism in the antioxidant function of vitamin E. Crit Rev Toxicol 23, 147–169, 1993.
  75. Altucci L, Gronemeyer H, Nuclear receptors in cell life and death, Trends Endocrinol Metab 12, 460–468, 2001.
  76. Ross SA, McCaffery PJ, Drager UC, De Luca LM, Retinoids in embryonal development, Physiol Rev 80, 1021–1054, 2000.
  77. Palace VP, Khaper N, Qin Q, Singal PK, Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease, Free Radic Biol Med 26, 746–761, 1999.
  78. Vega VA, Anzulovich AC, Varas SM, Bonomi MR, Gimenez MS, Oliveros LB, Effect of nutritional vitamin A deficiency on lipid metabolism in the rat heart: Its relation to PPAR gene expression, Nutrition 25, 828–838, 2009.
  79. Keser S, Celik S, Turkoglu S, Yilmaz O, Turkoglu I, Effects of *Rubus discolor* flower extracts on the vitamin D and K contents in hydrogen peroxide administered Wistar rats tissues, Firat Univ J Sci 25, 121-129, 2013.

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