



RESEARCH ARTICLE

Evaluation of the Erythrocyte Fragility, Haematological Parameters and Antioxidant Properties of *Platanus orientalis* Leaf Infusion Against Ethanol Toxicity in Rats

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ABSTRACT

The aim of this study was to evaluate the protective effects of the leaf infusion of chinar (*Platanus orientalis* L.) on erythrocyte fragility, haematological parameters and antioxidant status against ethanol-induced oxidative stress in rats. Thirty male rats were divided into five groups: Control, Ethanol, Ethanol+Silymarin (10 mg kg⁻¹), Ethanol+PO-20 mg mL⁻¹ infusion, and Ethanol+PO-60 mg mL⁻¹ infusion. According to the results, in the Ethanol group, erythrocyte counts, red cells distribution, plateletcrit, platelet and lymphocyte levels significantly decreased compared to the Control group, while PO-60 dose-fed group showed a significant increase in haematocrit and haemoglobin values compared to the Ethanol group. There were significant changes in erythrocyte fragility of Ethanol and Ethanol-treatment groups at different NaCl concentrations of 0.3 and 0.6 according to Control group. It was observed that PO Leaf infusion reduced the hemolysis caused by ethanol at a concentration of 0.3% NaCl, thus reducing the values to the control values. In addition, PO leaf infusion caused a significant increase in total antioxidant status against ethanol toxicity and a significant decrease in total oxidative status and oxidative stress index. It was concluded that PO leaf infusion may have antihematotoxic effect, reducing erythrocyte fragility and increase antioxidant capacity against ethanol toxicity.

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Introduction

Reactive oxygen species (ROS) or free radicals are substances generated by oxygen (O₂) metabolism which is balanced by the proportion of oxidant formation and the proportion of oxidant elimination (Sinha and Dabla, 2015). Oxidative stress (OS) results in the formation of free radicals or ROS due to insufficient antioxidant defence systems. There are many endogenous and exogenous factors causing OS. Ethanol (EtOH) is one of the exogenous factors causing OS. The majority of EtOH is metabolised in the liver by alcohol

dehydrogenase, aldehyde dehydrogenase and catalase (Zakhari, 2006; Pari and Suresh, 2008). The activation of O₂ by cytochromes P450 generates superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), which contributes to oxidative tissue damages (Lu and Cederbaum, 2008). Free radicals are among the by-products of EtOH metabolism and are known to cause cellular and tissue damage, unless the body can use antioxidants to clean them up (Varga et al., 2017). Ethanol-induced oxidative stress increases the production of ROS which cause damage to the RBC membrane via lipid peroxidation (Wrońska-Noferd et al., 1991). Additionally, it causes a deteriorating effect on the

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membrane integrity by increasing fluidity as well as the deactivation of membrane-bound receptors and enzymes (Arihan et al., 2016).

To the best of our knowledge, the effects of PO leaf infusion on hematological parameters have not been investigated so far. It was one of our main goals to uncover the possible effects of PO leaf infusion consumption in eliminating ethanol-induced hematological problems. Since, haematological parameters, erythrocytes, leukocytes and platelets, are commonly used to diagnose various diseases such as anemia, inflammatory, autoimmune, allergic, infectious, cancer, neutrophil and lymphocyte-related parameters as well as some further common disorders (Gao et al., 2019). The osmotic fragility test is one of the additional screening tests for evaluates the red blood cells ability to associate hypotonic solutions (King et al., 2015). The excess of hemolysis means that the structure of the cell membrane is destroyed.

Antioxidant mechanisms protect cells and tissues against free radicals. The total oxidative status (TOS), total anti-oxidative status (TAS) ratio and oxidative stress index (OSI) are indicative parameters of the degree of OS (Tamura et al., 2016). TAS and TOS are well known indicator parameters for the analysis of the oxidative stress in many diseases such as cancer, diabetes, cardiovascular and organ damage.

Platanus orientalis (Plantanaceae), also known as Chinar or Oriental plane, is commonly used in folk medicine against tooth and knee pain, wounds, inflammation, liver, kidney and stomach diseases (Dogan and Anuk, 2019). In previous studies it has been reported that *P. orientalis* contains important compounds such as kaempferol and kaempferol derivatives, caffeic acid, platanoside, tiliroside, flavonol and proanthocyanidin glycosides, phytol derivatives, benzaldehyde, palmitic acid, 2,4-ditert-butylphenol, stearic acid, octadecanoic acid, linoleic acid, and linolenic acid (Dogan and Anuk, 2019; Khan, 2017).

The purpose of this study was to determine the possible effects of PO-leaf infusion on the erythrocyte fragility, haematological parameters and antioxidant/oxidant capacity in erythrocyte of rats with ethanol-induced oxidative stress.

Materials and Methods

Plant Material and Preparation of the Infusion

The *Platanus orientalis* leaves were collected by Abdulhad Dogan in Haci Hamza hamlet, district of Dargeçit, city of Mardin, in the south-eastern Anatolian region of Turkey (GPS coordinates: 37° 33' 19.7"N; 41° 47' 43.3"E) in August, 2017. The identification of the samples was confirmed by Dr. Abdullah Dalar at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Van Yuzuncu Yil University, Turkey, and a voucher specimen was deposited in the university's herbarium (Herbarium code: 340 and Collector No: A.D-761, Van Yuzuncu Yil University Faculty of Pharmacy Herbarium).

The infusion of PO-leaf was prepared according to the study by Dogan and Anuk (2019). Briefly, the fresh PO- leaf samples were washed under tap water and dried at room temperature

in the dark until dryness. The powdered samples were kept in boiling water (100 °C) for about 2 min. Then, the heating was stopped and the ground leaves were allowed to remain in the water for about 15 min. Subsequently, the liquid in the container was first filtered through a gauze cloth (rough-hew) and then through a 0.45-µm hydrophilic filter (Millipore) using a disposable injector.

Chemicals

Ethanol, silymarin, adenosine, hydrogen peroxide solution (H₂O₂, %30), ammonium sulphate, phenol, o-dianisidine, ethylenediaminetetraacetic acid (EDTA), sodium hypochlorite solution, potassium dihydrogen phosphate (KH₂PO₄), sodium chloride (NaCl), and sodium dihydrogen citrate anhydrous (C₆H₇NaO₇) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). TAS and TOS kits were supplied by Rel Assay Diagnostics Laboratories Ltd.

Animals

The male rats (*Wistar albino*) of ~ 2 months of age and an average weight of 200 g were provided by the Experimental Animal Research Centre, Van Yuzuncu Yil University (Van, Turkey). The animals were housed at 25 ± 2 °C at a daily light/dark photoperiod of 10:14. All of the animals received water and a wheat-soybean-based diet *ad libitum* in stainless steel 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 Institute of Health. 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 Ethics Committee of Van Yuzuncu Yil University (Protocol number: 27552122-604.01.02-E.70881).

Experimental Design

After toxicity test, the rats were randomly divided into 5 groups, with each containing 6 rats and the study was continued for 28 days.

Control group: The rats were allowed to receive tap water and a standard pellet diet *ad libitum*.

Ethanol group: The rats were allowed to receive 20% ethanol and a standard pellet diet *ad libitum*. The dose of ethanol was selected on the basis of a 20% concentration that was administered orally, which caused OS (Dogan and Anuk, 2019).

Ethanol + Silymarin: The rats were allowed to receive 20% ethanol and silymarin (10 mg kg⁻¹, single dose per day) and were treated orally during the experimental period.

Ethanol + PO-20 group: The rats were allowed to receive 20% ethanol and *P. orientalis* (20 mg mL⁻¹) leaf infusion during the experimental period.

Ethanol + PO-60 group: The rats were allowed to receive 20% ethanol and *P. orientalis* (60 mg mL⁻¹) leaf infusion during the experimental period.

Measurement of Erythrocyte Fragility

Blood samples were incubated for 24 hours at room temperature. 30 μL of the samples with erythrocytes were added to solutions containing NaH_2PO_4 and Na_2HPO_4 buffers in order to achieve suitable pH and NaCl concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9%. After 30 minutes of incubation at room temperature, the solutions were centrifuged at 3000 rpm for 5 minutes. Absorbance of supernatant fractions was evaluated with a spectrophotometer at 546 nm (Dogan, 2018).

Calculation: Haemolysis (%) = (OD of Test Solution)/(OD of Standard Solution) \times 100

Haematological Parameters

At the end of the 28 days of experiment, blood samples were obtained the cardiac puncture by using syringe for the determination of hematological constituent. Red blood cells counts (RBC), red cells distribution width (RDW), haematocrit (HCT), haemoglobin (HGB), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), plateletcrit (PCT), platelet (PLT), platelet distribution width (PDW), platelet large cell ratio (PLCR), white blood cell (WBC), lymphocyte (LYM), monocytes (MON) and granulocyte (GRA) were measured by an automatic hematological assay analyzer (Coulter LH 780 Analyzer, US).

Measurement of TOS, TAS and OSI Parameters

The levels of TOS in plasma were assessed spectrophotometrically at 530 nm using kits developed by Erel (2005). The TAS levels in plasma were measured spectrophotometrically (AE-S90-MD UV/VIS spektrofotomet) at 660 nm using kits developed by Erel (2004). The percent ratio of TOS to TAS level was considered as the oxidative stress index (OSI) and this value was calculated according to the study by Tülüce et al (2017).

Statistical Analysis

All of the obtained data were expressed as the mean \pm standard deviation (SD). The statistical analyses were made using the Minitab 14 packet program for Windows. The one-

way analysis of variance (ANOVA) was used to determine the differences between the means of the experimental groups and p value \leq 0.05) was considered statistical significance.

Results

PO-Leaf Infusion Effects on the Erythrocyte Osmotic Fragility

As shown in Table 1 and Figure 1, there were no significant differences in the degree of erythrocyte hemolysis of groups at NaCl concentrations of 0.1, 0.2, 0.8 and 0.9 g L^{-1} . However, there were significant changes in erythrocyte fragility of Ethanol, silymarin and PO-treatment groups at NaCl concentrations of 0.3, 0.6 and 0.7 (Ethanol and Ethanol + PO-20 group) mg mL^{-1} compared to the Control group. However, at the NaCl concentration of 0.3 Ethanol-treated groups (silymarin and PO-leaf infusion doses) exhibited a significant decrease compared to the Ethanol group.

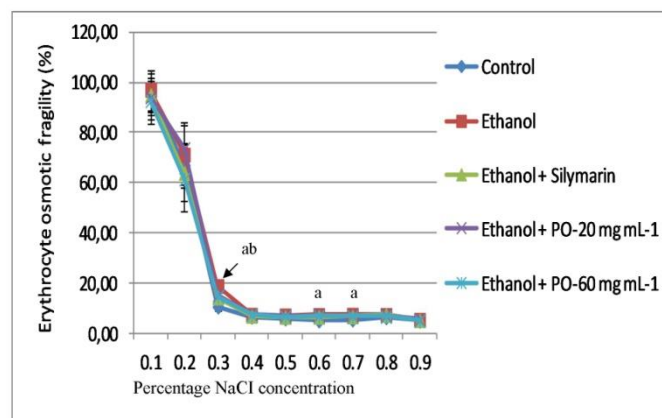


Figure 1. Effects of silymarin and PO-leaf infusion on the erythrocyte osmotic fragility against ethanol-induced oxidative stress in rats [Data were expressed as the mean \pm SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups). ^a Difference between the Control group and the other groups was significant (p \leq 0.05). ^b Difference between the Ethanol group and the other groups was significant (p \leq 0.05)]

Table 1. Effects of silymarin and PO-leaf infusion on the erythrocyte osmotic fragility against ethanol-induced oxidative stress in rats

Groups	Percentage NaCl concentration									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	
Control	95.35 \pm 8.08	67.06 \pm 8.94	10.54 \pm 1.33	6.51 \pm 0.93	5.82 \pm 1.00	5.01 \pm 0.24	5.39 \pm 0.81	6.31 \pm 0.73	4.86 \pm 0.28	
Ethanol	97.07 \pm 7.98	71.21 \pm 11.81	18.81 \pm 1.65 ^a	7.37 \pm 0.49	7.08 \pm 0.93	7.23 \pm 0.44 ^a	7.36 \pm 0.48 ^a	7.30 \pm 0.74	5.37 \pm 0.71	
Ethanol + Silymarin	95.04 \pm 9.99	63.90 \pm 11.32	14.20 \pm 2.65 ^{ab}	6.88 \pm 0.71	6.49 \pm 0.58	6.53 \pm 0.70 ^a	6.67 \pm 0.76	7.26 \pm 0.64	5.15 \pm 0.29	
Ethanol + PO-20 mg mL^{-1}	94.21 \pm 6.23	73.63 \pm 10.55	15.28 \pm 0.82 ^{ab}	7.39 \pm 0.51	6.78 \pm 0.57	7.05 \pm 0.35 ^a	7.00 \pm 0.22 ^a	7.18 \pm 0.24	5.43 \pm 0.69	
Ethanol + PO-60 mg mL^{-1}	92.69 \pm 9.27	61.40 \pm 12.81	14.66 \pm 1.33 ^{ab}	7.20 \pm 0.42	6.58 \pm 1.07	6.76 \pm 0.66 ^a	6.67 \pm 0.75	6.61 \pm 0.31	5.00 \pm 0.34	

Data were expressed as the mean \pm SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups)

^a Difference between the Control group and the other groups was significant (p \leq 0.05).

^b Difference between the Ethanol group and the other groups was significant (p \leq 0.05).

^c Difference between the Ethanol + Silymarin group and the other groups was significant (p \leq 0.05).

^d Difference between the Ethanol + PO-20 mg mL^{-1} group and the Ethanol + PO-60 mg mL^{-1} group was significant (p \leq 0.05).

PO-Leaf Infusion Effects on the Haematological Parameters

The levels of the erythrocyte and platelet parameters were given in Table 2. The levels of RBC and RDW in the Ethanol group were significantly increased compared to the Control group; however, the HCT and HGB levels in the Ethanol + PO-leaf 60 group were significantly increased compared to the Ethanol group. The levels of PCT and PLT in the all groups were significantly reduced compared to the Control group. MPV and PDW values were significantly decreased in Ethanol + Silymarin group compared to Ethanol + PO treated groups. Additionally,

PDW and LPCR levels in the Ethanol + Silymarin group were significantly lower than Ethanol group.

The levels of WBC, LYM, MON and GRA were given in Table 3. The LYM levels in Ethanol group were significantly lower than Control group. MON (%) and GRA (%) levels in Ethanol + Silymarin group were significantly higher than Control group. Additionally, the MON ($10^9 L^{-1}$) and GRA ($10^9 L^{-1}$) levels in the Ethanol + Silymarin group were significantly increased compared to the almost all groups. The level of GRA (%) in the ethanol group was significantly increased compared to all other groups except for the Ethanol + PO-20 group.

Table 2. Effects of silymarin and PO-leaf infusion on the on erythrocytic and platelets parameters against ethanol-induced oxidative stress in rats

Parameters	GROUPS				
	Control	Ethanol	Ethanol + Silymarin	Ethanol + PO-20 mg mL ⁻¹	Ethanol + PO-60 mg mL ⁻¹
RBC ($10^{12} L^{-1}$)	8.69 ± 0.53	7.82 ± 0.61 ^a	8.36 ± 0.39	8.48 ± 0.45	8.35 ± 0.66
RDW (%)	15.10 ± 0.42	13.53 ± 0.68	14.32 ± 0.45	14.88 ± 0.89	14.68 ± 0.99
RDW _a (fL)	33.76 ± 0.34	31.70 ± 1.94 ^a	33.00 ± 1.43	33.36 ± 1.52	31.98 ± 2.06
HCT (%)	48.22 ± 2.88	44.78 ± 2.61	47.36 ± 2.97	47.22 ± 2.29	48.75 ± 1.35 ^b
HGB (g dL ⁻¹)	15.72 ± 0.91	14.87 ± 0.50	15.36 ± 0.78	15.40 ± 0.76	15.80 ± 0.16 ^b
MCH (pg)	18.12 ± 0.43	18.50 ± 0.37	18.40 ± 0.07	18.20 ± 0.35	18.98 ± 1.27
MCHC (g dL ⁻¹)	32.66 ± 0.49	32.85 ± 1.00	32.54 ± 0.47	32.68 ± 0.36	33.02 ± 1.54
MCV (fL)	55.42 ± 0.62	56.40 ± 1.12	56.43 ± 0.70	55.76 ± 0.95	55.50 ± 2.03
MPV (fL)	6.52 ± 0.24	6.58 ± 0.13	6.36 ± 0.18	6.58 ± 0.11 ^c	6.64 ± 0.21 ^c
PCT (%)	0.49 ± 0.09	0.28 ± 0.05 ^a	0.32 ± 0.04 ^a	0.33 ± 0.03 ^a	0.30 ± 0.04 ^a
PLT ($10^9 L^{-1}$)	689.00 ± 54.25	445.50 ± 57.25 ^a	508.00 ± 40.55 ^a	496.60 ± 59.62 ^a	458.00 ± 41.04 ^a
PDW (fL)	8.46 ± 0.23	8.53 ± 0.13	8.26 ± 0.18 ^b	8.48 ± 0.11 ^c	8.56 ± 0.21 ^c
LPCR (%)	5.08 ± 1.03	5.88 ± 0.90	4.42 ± 0.61 ^b	5.66 ± 0.76	5.68 ± 1.16

Data were expressed as the mean ± SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups)

^a Difference between the Control group and the other groups was significant (p ≤ 0.05).

^b Difference between the Ethanol group and the other groups was significant (p ≤ 0.05).

^c Difference between the Ethanol + Silymarin group and the other groups was significant (p ≤ 0.05).

RBC: Red Blood Cells counts; RDW: Red cells Distribution Width; HCT: Haematocrit; HGB: Haemoglobin; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume; MPV: Mean Platelet Volume; PCT: Plateletcrit; PLT: Platelet; PDW: Platelet Distribution Width; PLCR: Platelet Large Cell Ratio.

Table 3. Effects of silymarin and PO-leaf infusion on the different leukocyte parameters against ethanol-induced oxidative stress in rats

Parameters	GROUPS				
	Control	Ethanol	Ethanol + Silymarin	Ethanol + PO-20 mg mL ⁻¹	Ethanol + PO-60 mg mL ⁻¹
WBC ($10^9 L^{-1}$)	3.73 ± 0.75	4.60 ± 1.20	5.04 ± 1.08	4.12 ± 0.72	5.20 ± 1.12
LYM ($10^9 L^{-1}$)	4.23 ± 1.06	3.28 ± 0.71	3.74 ± 0.90	3.12 ± 0.87	3.58 ± 0.43
LYM (%)	82.23 ± 0.94	75.93 ± 4.10 ^a	74.38 ± 7.98	80.54 ± 4.69	81.68 ± 3.87
MON ($10^9 L^{-1}$)	0.35 ± 0.06	0.33 ± 0.10	0.60 ± 0.16 ^{ab}	0.38 ± 0.08 ^c	0.35 ± 0.06 ^c
MON (%)	8.60 ± 1.63	8.93 ± 1.68	11.16 ± 1.87 ^a	8.12 ± 1.43 ^c	8.65 ± 1.98
GRA ($10^9 L^{-1}$)	0.33 ± 0.05	0.40 ± 0.08	0.62 ± 0.13 ^{ab}	0.46 ± 0.05 ^{ac}	0.43 ± 0.06 ^a
GRA (%)	9.68 ± 1.54	15.15 ± 3.23 ^a	14.46 ± 3.17 ^a	11.34 ± 2.58	9.68 ± 1.90 ^{bc}

Data were expressed as the mean ± SD. One-way ANOVA followed by the Tukey test, when appropriate (n= 6 animals for each of the 5 groups)

^a Difference between the Control group and the other groups was significant (p ≤ 0.05).

^b Difference between the Ethanol group and the other groups was significant (p ≤ 0.05).

^c Difference between the Ethanol + Silymarin group and the other groups was significant (p ≤ 0.05).

WBC: White Blood Cell; LYM: Lymphocyte; MON: Monocytes; GRA: Granulocyte.

PO-Leaf Infusion Effects on the TOS, TAS and OSI Parameters

The effects of TOS, TAS and OSI in plasma were presented in the Figure 2. The plasma level of TOS in the Ethanol group was significantly higher than in the Control and Ethanol + PO-treated groups. Furthermore, TOS activity in plasma showed a significant decrease in the Ethanol + PO-60 group compared to

the Ethanol + PO-20 group. On the other hand, Ethanol and Ethanol + silymarin group were decreased significantly compared to the control group, similarly in TAS levels. The Ethanol group showed a significant decrease compared to the Ethanol + PO-20 group. OSI levels in Ethanol, Ethanol + Silymarin and Ethanol + PO-20 groups were significantly decreased compared to the control group while OSI levels in

Ethanol group were significantly increased compared to Ethanol + PO-treatment groups and OSI levels of Ethanol + PO-60 administration group were significantly reduced compared to the Ethanol + PO-20 group.

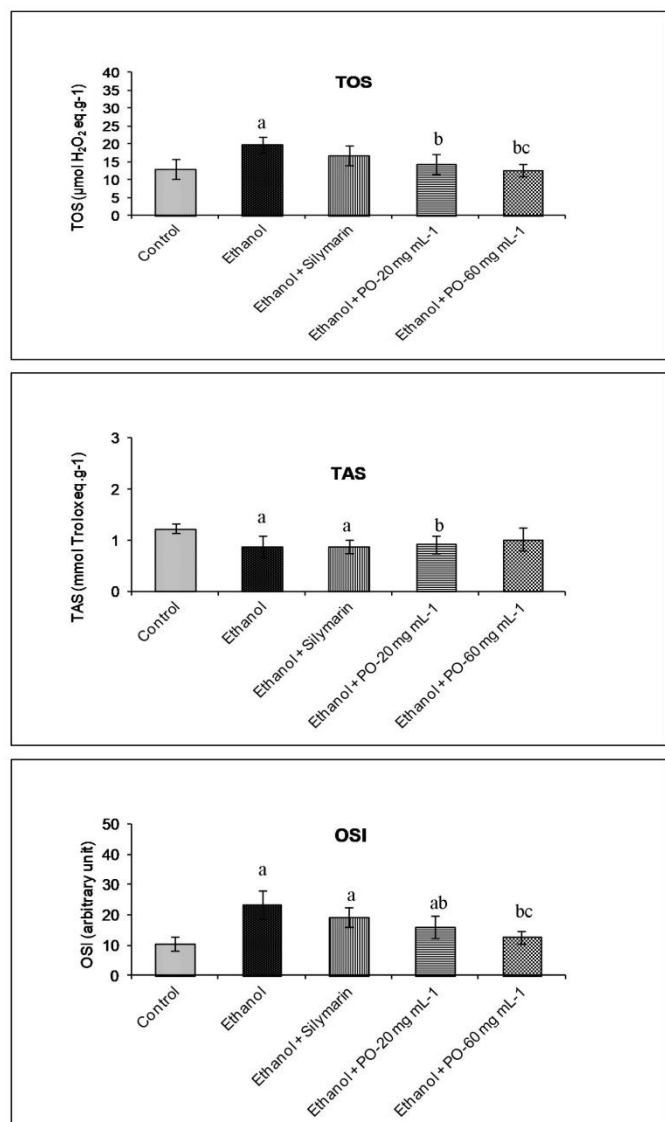


Figure 2. PO-leaf infusion effects on the TOS, TAS and OSI parameters against ethanol-induced oxidative stress in rats [Data were expressed as the mean \pm SD. One-way ANOVA followed by the Tukey test, when appropriate ($n = 6$ animals for each of the 5 groups). ^a Difference between the Control group and the other groups was significant ($p \leq 0.05$). ^b Difference between the Ethanol group and the other groups was significant ($p \leq 0.05$). ^c Difference between the Ethanol + Silymarin group and the other groups was significant ($p \leq 0.05$). ^d Difference between the Ethanol + PO-20 mg mL⁻¹ group and the Ethanol + PO-60 mg mL⁻¹ group was significant ($p \leq 0.05$)]

Discussion

Different parts of *Platanus orietalis* have been used in various diseases for many years worldwide. For example; various researchers have reported that the use of PO-leaves in folk medicine such as in ophthalmia (Haider et al., 2012) as well as in liver, kidney damages and obesity (Dogan and Anuk,

2019). Chopra et al. (1956) have reported that the use of PO-barks boiled in vinegar for toothache, diarrhea and dysentery. Another study has reported the use of PO-buds for urinary tract antimicrobial and antiseptic agents (Mitrokotsa et al., 1993), Platanoside and Tiliroside isolated from PO-fruits have been used for anti-aging and cell toxicity (Chatzigeorgiou et al., 2017). However, the effects of PO-leaf infusion on the erythrocyte fragility, haematological and TOS/TAS index parameters have not been investigated against ethanol-induced OS in experimental rats model. In addition, silymarin was used as a positive control group; because of preventing effect of on liver damage caused by various toxic substances such as ethanol and carbon tetrachloride (Dogan and Anuk., 2019; Shaker et al., 2010).

EtOH is used to create toxicity models in rats. Chronic alcohol consumption leads to liver and kidney damage and an increase in biochemical parameters such as gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine, lipid peroxidation and decreases in antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase (Dogan and Celik, 2012; Bati et al., 2015). EtOH can cause the suppression of the immune system and the development of various infections. The previous study showed that EtOH suppressed several leukocyte functions, phagocytic function of circulating neutrophils, as well as other neutrophil functions including adhesion, chemotaxis and oxygen metabolism (Chiu et al., 2018). Increased oxidative stress caused by EtOH leads to a decrease in reduced glutathione (GSH) levels, which has a significant protective role in erythrocytes against to protein thiol oxidation. This negative condition is responsible for decreased resistance to hemolysis (Padmini and Sundari, 2008). The EtOH, a highly cytotoxic chemical, has been reported to be responsible for the oxidation of proteins, erythrocyte abnormalities and hemolysis (Tyulina et al., 2006). It has also been reported that RBCs, membrane lipids, are very sensitive to metabolites that induce oxidation of proteins and increase fragility and this fragility is further increased in ethanol consumption (Alimi et al., 2012). In the present study, we evaluated for the first time the effect of PO-leaf infusion against ethanol-induced OS and osmotic fragility of rats erythrocytes. PO-leaf infusions against EtOH toxicity showed significant changes in hemolysis especially at 0.3 NaCl % concentration. We assume that this protective property may be a result of the effect of the plant content. In our previous study, we have reported that PO-leaf contains a variety of natural antioxidants including kaempferol and kaempferol derivatives, benzaldehyde, palmitic acid, 2,4-ditert-butylphenol, stearic acid, octadecanoic acid, linoleic acid and linolenic acid (Dogan and Anuk, 2019). The extracts containing kaempferol or kaempferol derivatives have been shown to inhibit erythrocyte hemolysis (Olchowik et al., 2012; da Cunha et al., 2016). These results were similar to the results obtained in this study.

The TOS, TAS and TOS/TAS index are important indicator parameters for the assessment of oxidative status. It has been reported that ethanol causes a decrease in TAS and an increase in TOS (Ozkol et al., 2017; Erkec et al., 2018). The cause of

this effect is uncertain, but is probably the result of various kaempferol and fatty acids contents in the plant.

Conclusion

When all of the obtained data were evaluated, it was determined that *P. orientalis* leaves possess antioxidant properties and protective effects on erythrocyte fragility and various haematological parameters against ethanol-toxicity in rats. We are planning to evaluate the effects of PO leaf infusion immunotoxicity and neurotoxicity in further studies.

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