Investigation of Neurotoxic and Immunotoxic Effects of the Chinar (Platanus orientalis L.) Tree Leaf Infusion Against Ethanol Toxicity in Rats

Abdulahad DOGAN1,*, Fatih DONMEZ1, Abdulhamit BATTAL2, Ali ASLAN3–4, Ozgur Ozan ANUK1

ABSTRACT: In this study, immunotoxic and neurotoxic effects of leaf infusion of Platanus orientalis L. (PO) were investigated in rats ethanol-induced toxicity. Neurotoxic effects of ethanol were evaluated by measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in rat brain tissue. Immunotoxic effects were evaluated by adenosine deaminase (ADA) and myeloperoxidase (MPO) biomarkers in rat liver, lung, erythrocyte and spleen tissues. Thirty male Wistar rats were divided into five groups after toxicity study. The groups are explained as follow: Control, Ethanol 20 %, Ethanol 20 % + Silymarin (10 mg kg⁻¹), Ethanol 20 % + PO-20 mg mL⁻¹ infusion and Ethanol 20 % + PO-60 mg mL⁻¹ infusion. The results showed that AChE and BChE activities statistically decreased in the groups treated with PO leaf infusion. While there was a statistically significant decrease in ADA and MPO activities in liver tissue of groups treated with PO leaf infusion. Also, MPO activity of erythrocyte in ethanol group was significantly increased according to silymarin and PO-60 groups. On the other hand, there was no statistically significant finding was found in lung and spleen tissues. It was concluded that the infusion prepared from P. orientalis leaves suppressed to ethanol-induced neurotoxicity and immunotoxicity.

Keywords: Platanus orientalis, acetylcholinesterase, butyrylcholinesterase, adenosine deaminase, myeloperoxidase, ethanol.

1 Abdulahad DOGAN (Orcid ID: 0000-0002-5438-8560), Fatih DONMEZ (Orcid ID: 0000-0003-3958-1028), Ozgur Ozan ANUK (Orcid ID: 0000-0002-9028-7118), Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Biochemistry, Van, Turkey.
2 Abdulhamit BATTAL (Orcid ID: 0000-0001-6098-3908), Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey.
3,4 Ali ASLAN (Orcid ID: 0000-0002-2637-4786), Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology, Van, Turkey or Kyrgyz-Turkish Manas University, Faculty of Arts and Science Department of Biology, Bishkek, Kyrgyzstan.

*Corresponding Author: Abdulahad DOGAN, e-mail: abdulahaddogan@yyu.edu.tr

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INDRODUCTION

Alcoholism is associated with cognitive deficits and loss of brain mass that is documenting neuron and myelin loss (Nixon and Crews, 2002). Ethanol is a widely used for production of beverages, as an organic solvent in laboratory and creating toxicity models for experimental animals. Its moderate consumption by people can lead to damage to both the nervous system and the immune system (Chardwick and Sisson, 2015; Hosseini et al., 2017). The underlying neurotoxic effects of ethanol can be explained by pro-oxidant effects and disturbances in antioxidant defense mechanisms (Mitchell et al., 1999). Cholinesterase activity is a biological marker of neurotoxicity (Olson, 2018). Cholinesterases (ChE) are enzymes that hydrolyze acetylcholine (ACh) to choline and acetic acid. There are two types of cholinesterase: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) (Gülçin et al., 2016). Similarly, adenosine deaminase (ADA; EC 3.5.4.4) and myeloperoxidase (MPO; EC 1.11.2.2) are biomarkers of the immune system in vertebrates (Gulec et al., 2006; Celik et al., 2010). ADA is an enzyme that catabolizes adenosine or deoxyadenosine and MPO catalyzes the formation of hypochlorous acid or hypothiocyanite (Tamura et al., 2016; Lu et al., 2018).

Silymarin is a liver protective bioactive compound isolated from seeds of milk thistle (Silybum marianum L.) belong to Asteraceae family (Demartini and Esposti, 2002). Silymarin has been used extensively against toxic chemicals, alcohol-related fat infiltration, gallbladder diseases, toxin and fungal intoxications, various liver diseases such as hepatitis and snake and insect bites for 2000 years due to its protective effect on liver (Kocaman and Özlem, 2015).

Platanus orientalis L. (PO) is a perennial tree species of the Platanaceae family and in Turkish folk medicine, this plant is known as “çınar” (Mitrokotsa et al.,1993; Tuzlacı and Erol, 1999). Since ancient times, Platanus orientalis has been used as a painkiller against dental pain and knee pain and inflammation in folk medicine (Hajhashemi et al., 2011). Furthermore, the effects of PO infusion and extracts and compounds isolated from PO were investigated in anticancer, anti-inflammatory and antiseptic, oxidative stress, toothache and dermatological and rheumatic diseases or disorders (Tantry et al., 2012; Haider et al., 2012; Shende et al., 2018; Khan, 2017; Dogan and Anuk, 2019). In spite of modern medicine, the use of complementary and alternative medicine is increasing worldwide and many medicinal plants are being used for disease treatment and health improvement (Eardley et al., 2012). In the present study we investigated the effects of P.orinetalis leaf infusion on neurotoxicity and immunotoxicity against ethanol-induced toxicity in rats.

MATERIALS AND METHODS

Chemicals

The ethanol, silymarin, Acetylthiocholine iodide (AChI), butyrylthiocholine iodide (BChI), 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), potassium phosphate (KH₂PO₄), adenosine, phenol, sodium hydroxide, sodium nitroprusside, ammonium sulfate, sodium hypochlorite, hexadecytrimethylammonium bromide and o-dianisidine dihydrochloride of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

Plant Material and Preparation of the Infusion

The P. orientalis leaves were collected from 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 herbarium (Herbarium code: 340 and Collector No: A.D-761, Van Yuzuncu Yil University Faculty of Pharmacy Herbarium).

The PO leaf infusion was prepared according to Dogan and Anuk (2019). The fresh *P. orientalis* leaf samples were washed under tap water and dried at room temperature in the dark until dry. The powdered samples were kept in boiling water (100 °C) for about 2 min. Next, 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 an injector.

**Animals**

Male Wistar albino rats of approximately 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). They were divided into five groups, with each group containing six rats. The animals were housed at 25 ± 2 °C at a daily light/dark photoperiod of 10:14-1. All of the animals were given a wheat-soybean-based diet and water 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 ethical regulations followed were 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**

The rats were randomly divided into five groups, with each containing six rats. 

Control group: The rats received tap water and a standard pellet diet *ad libitum.*

Ethanol group: The rats received 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 oxidative stress (Dogan and Anuk, 2019).

Ethanol + Silymarin: The rats received 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 received 20% ethanol and *P. orientalis* (20 mg mL⁻¹) leaf infusion during the experimental period.

Ethanol + PO-60 group: The rats received 20% ethanol and *P. orientalis* (60 mg mL⁻¹) leaf infusion during the experimental period (Dogan and Anuk, 2019).

**Preparation of the Tissue Supernatants**

At the end of the 28 days experiments, the rats were anesthetized via an injection of ketamine (5 mg (100 g)⁻¹ of body weight), intraperitoneally. The brain, liver, lung and spleen tissues were dissected and put into Petri dishes. Subsequently, the samples were taken and kept at −78 °C until analysis. The tissues were homogenized for 5 min in 50 mM of ice-cold potassium dihydrogen phosphate (KH₂PO₄) solution (1:5 w⁻¹) using a stainless steel probe homogenizer (SONOPULS HD 2200, Bandelin, Berlin, Germany), and then subsequently centrifuged at 7000 ×g for 15 min. All of the processes were carried out at 4 °C. In addition, the erythrocyte were washed three times with physiological saline (0.9 % NaCl). Then, erythrocyte package and supernatants were used to determine neurotoxicity and immunotoxicity parameters (Celik et al., 2011).
Biochemical Analysis

AChE and BChE activities were measured on AE-S90-MD UV/VIS spectrophotometer using acetylthiocholine iodide and butyrylthiocholine iodide as substrate, respectively, by the method of Ellman et al. (1961). ADA was assayed according to Giusti and Bergmeyer (1974). MPO was assayed by the method described by Bradley et al. (1982).

Statistical Analysis

GraphPad Prism software (version 6; GraphPad Inc., U.S.A) was used for data analysis which all were presented as mean ± standard error of mean (SEM). The significance of difference between groups were determined using one-way analysis of variance (ANOVA) and Tukey tests. p < 0.05 and p < 0.01 was considered to be significantly different.

RESULTS AND DISCUSSION

Throughout the history of humanity, plants have been used in the treatment of many diseases (Gurib-Fakim, 2006). Platanus orientalis L., a medicinal tree from the family Platanaceae, is one of the largest and longest-lived trees in the Eastern Mediterranean (Hajhashemi et al., 2011; Rix ve Fay, 2017). Anticancer, antiinflammatory and antinociceptive activities of P. orientalis and its effects in asthma, ulcerogenic risk assessment, hoarseness and its use as anti-aging have been reported up to now (Tantry et al., 2012; Haider et al., 2012; Asadbeigi et al., 2014; Chatzigeorgiou et al., 2017). Ethanol have toxic effects on the immune system and nervous system (Brust, 2010; Chardwick and Sisson, 2015). Medicinal plants and their bioactive compounds are using against immunotoxic and neurotoxic damage induced by chemicals, pesticides and heavy metals. The protective role of quercetin, which is an important bioactive compound mostly derived from plants, on the immunotoxic and neurotoxic effects was investigated in cadmium-exposed rats. Reductions in ADA, MPO and AChE activities were observed when quercetin groups were compared with cadmium exposed groups (Abdalla et al., 2014). AChE and BChE activities for neurotoxicity in brain and ADA and MPO activities for immunotoxicity in liver, lung, erythrocyte and spleen tissues were evaluated in the study. Neurotoxicity and immunotoxicity activities of PO leaf infusion were also investigated for the first time in rats induced by ethanol toxicity.

Effects of PO Tree Leaf Infusion on Neurotoxicity Against Ethanol Toxicity in Rats Brain Tissue

As shown in Figure 1, the AChE activity in brain tissue was increased in ethanol group compared to all other groups. 60 mg mL⁻¹ PO leaf infusion caused significantly decrease in BchE activity in brain tissue according to the ethanol group.

In this study, neurotoxicity and immunotoxicity activities of PO leaf infusion were investigated for the first time in rats induced by ethanol toxicity. When the results were evaluated; AChE and BChE activities significantly decreased in brain tissue of PO treated groups compared to the ethanol group.

Enzymes metabolizing ChE have important roles in the body (Bilgi et al., 2003). ChE’s are naturally found in many organisms from microorganisms to vertebrates and they play a crucial role in transmission of nervous influx (Jebali et al., 2013). AChE is found in nerve endings, erythrocytes, spleen, lungs and all parts of the brain; BChE is found in the heart, smooth muscles, liver, intestines, pancreas, and white matter of the brain (Patočka et al., 2004). Oxygen-induced free radicals cause neurotoxicity and consequently reduce ChE enzyme activities (Mitchell et al., 1999; Celik et al., 2011). It was reported that hydrogen sulfide had a therapeutic potential against neurotoxicity induced
by alcohol (Gezginci-Oktayoglu et al., 2014; George et al., 2017). Our findings are consistent with previous studies that PO leaf infusion caused reduction on the neurotoxic effects of ethanol.

**Figure 1.** Effects of PO Tree Leaf Infusion on AChE and BChE Enzymes Against Ethanol Toxicity in Rat Brain Tissue

All of the data were expressed as the mean ± standard error of mean (SEM). One way ANOVA followed by Tukey test, when appropriate (n=6). * Significant difference (P <0.05), ** Significant difference (P <0.01)

**Effects of PO Tree Leaf Infusion on Immunotoxicity Against Ethanol Toxicity in Some Rat Tissues**

The ADA activities of Ethanol 20 % and Ethanol 20 % + PO-20 mg mL⁻¹ infusion groups in liver tissues were significantly increased compared to the control (Figure 2). On the contrary there was no statistically difference for the ADA activities in lung, erythrocyte and spleen tissues (Figure 2).

While ethanol caused increase in MPO activity in liver compared to control group, MPO activity significantly decreased in 60 mg mL⁻¹ PO leaf infusion treated group, similar activity with control. However, MPO activity of 20 mg/mL PO leaf infusion treatment was similar to ethanol group (Figure 3). MPO acitivity in erythrocyte significantly decreased in silymarin and 60 mg mL⁻¹ PO leaf infusion treated groups compared to ethanol group (Figure 3). There was no statistically difference for the MPO activities in lung and spleen tissues.

According to our results, while 60 mg mL⁻¹ of PO leaf infusion caused significant decreases in ADA and MPO activities in liver tissue compared to ethanol group. The immune system is highly susceptible to degradation by chemical agents and toxic substances especially ethanol (Tonk et al., 2013). Increase in ADA and MPO activities were reported in the inflammation (Abdalla et al., 2014; Meisel et al., 2002). Ethanol causes increase in the amounts of free radicals and results with increasing ADA and MPO activity (Nagy et al., 1990). While ethanol caused increase in MPO levels in brain and liver tissues and ADA levels in liver tissue, treatment compounds tested against ethanol toxicity in rats caused decrease in these enzyme levels (Ozkol et al., 2017). It was reported that ADA levels in serum were increase in hepatit C virus infected patients and suffering from liver insufficiency patients, especially ADA₂ which is a major isoenzyme (Fernández et al., 2000). The protective effect of
Syzygium cumini leaf extract was reported against ethanol-induced acute injury in rats by inhibiting ADA activity in serum and lymphocytes (Cargnelutti et al., 2015). Our findings are consistent with previous studies that PO leaf infusion reduced the immunotoxicity caused by ethanol.

**Figure 2.** Effects of PO Tree Leaf Infusion on ADA Enzyme Against Ethanol Toxicity in Some Rat Tissues
All of the data were expressed as the mean ± standard error of mean (SEM). One way ANOVA followed by Tukey test, when appropriate (n=6). * Significant difference (P <0.05), ** Significant difference (P <0.01)
Figure 3. Effects of PO Tree Leaf Infusion on MPO Enzyme Against Ethanol Toxicity in Some Rat Tissues
All of the data were expressed as the mean ± standard error of mean (SEM). One way ANOVA followed by Tukey test, when appropriate (n=6). * Significant difference (P <0.05), ** Significant difference (P <0.01).

CONCLUSION

As a conclusion, when AChE and BchE activities in brain and MPO and ADA activities in liver and erythrocyte were evaluated, *ad libitum* intake of 60 mg mL⁻¹ of *P. orientalis* leaf infusion had positive effects on immunotoxic and neurotoxic effects of ethanol toxicity in rats. As a further study, bioactive compounds of PO should be isolated and their individuals effects should be evaluated against ethanol toxicity. Additionally, molecular mechanisms of PO and ethanol toxicity interaction should be evaluated.
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