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Cotinus coggygria Scop.: A study on the Investigation of in vitro Antioxidant Activity and Prevention of Ethanol-induced Rat Brain Damage

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ABSTRACT

Cotinus coggygria Scop. (*C. coggygria*) has been reported to contain numerous secondary metabolites. In the present study, *in vitro* total phenolic and flavonoid contents and antioxidant activity of *C. coggygria* extract were determined. In *in vivo* experiments, rats were divided into four groups. Total oxidant and antioxidant status, nitric oxide, reduced glutathione, lipid peroxidation, sialic acid, advanced oxidized protein product levels and carbonic anhydrase, aryl esterase, xanthine oxidase, lactate dehydrogenase, paraoxonase, prolidase, acetylcholinesterase enzyme activities and antioxidant and glutathione-related enzyme activities were determined in all brain tissue homogenates. According to the results, aqueous *C. coggygria* extract shows protective properties against ethanol-induced brain damage due to various bioactive components it contains and provides restoration of biochemical parameters. Considering the data from our study, *C. coggygria* can be used to prevent ethanol-induced oxidative stress in brain tissue due to its strong antioxidant activity.

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Keywords: *Antioxidant activity, Brain, Cotinus coggygria Scop., Ethanol, Oxidative stress.*

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1. Introduction

Herbal medicine is an important tool and alternative to the treatment of several diseases. The secondary metabolites produced by plants play a significant part in protecting human health. They include a range of bioactive substances, such as terpenoids, flavonoids, alkaloids, tannins, and polyphenols, particularly derivatives of gallic acid that function as antioxidants. The plant known as a smoke tree, *Cotinus coggygria* Scop. (*C. coggygria*) (Anacardiaceae), is utilized in Southeast Europe in traditional folk medicine because of the antibacterial, anti-inflammatory, antiviral, antifungal, hepatoprotective, and antigenotoxic properties (Figure 1). Because it contains a variety of anthocyanins, chalcones, aurones, flavonoids, and other secondary polyphenolic metabolites, it has also significant medicinal potential on the modulation of the epidermal growth factor receptor (EGFR) activity by inhibition of its oncogenic signaling in brain cancer cells [1].

Alcohol use disorder, a very common and life-threatening condition with severe adverse health effects, is a significant reason for morbidity and mortality worldwide. When an organism is exposed to either acute alcohol intoxication or chronic alcohol use, acetaldehyde and other metabolites accumulate in the body because of the decomposition of ethanol. As a result, several reactive oxygen species (ROS) form, which can cause cancer, heart and liver diseases, and other illnesses that impact the organism's central nervous system [2].



Figure 1. *Cotinus* coggygria Scop.plant

Besides oxidative stress and inflammation, other mechanisms involved in alcohol-induced liver injury include gut barrier dysfunction, endotoxin-mediated immune activation, mitochondrial damage, dysregulated lipid metabolism, impaired autophagy, and hepatic stellate cell activation leading to fibrosis. These processes interact with each other and collectively contribute to disease progression.

The brain tissue contains antioxidant markers enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). The

production of ROS and antioxidant enzymes remains in balance under a normal physiological condition. However, oxidative stress takes place when this balance is disrupted because of overproduction of ROS [3]. Alcohol consumption can damage central and peripheral nervous system and alcohol-induced oxidative stress can cause neuronal death, blood brain barrier damage, neurotoxic injury and neuroinflammation by accumulation of iron in specific regions of the brain [4].

In this study, our first objective is to determine the *in vitro* antioxidant activity of the *C. coggygria* plant along with its total phenolic and flavonoid content. Our second objective is to investigate whether this aqueous plant extract has a protective effect against ethanol-induced brain damage.

2. Materials and Methods

2.1 Plant Collection and Extract Preparation

C. coggygria leaves were obtained from the Bartın region of Türkiye. The plant was identified by Şükran Kültür, a faculty member at the Istanbul University Faculty of Pharmacy (ISTE No: 93133).

The leaves were washed and dried at room temperature. Approximately 50 g of leaves and 500 mL of distilled water were placed in a glass flask and boiled for 8 hours. The resulting solution was lyophilized, yielding a 29% extract.

An aqueous solution was prepared by dissolving the extract in distilled water and was used to determine its antioxidant activity, total phenolic and total flavonoid contents, as well as its protective effect against ethanol-induced brain damage.

2.2 Assay for *In Vitro* Antioxidant Activity

2.2.1 $ABTS^{\cdot+}$ radical scavenging activity assay

The method was applied according to Arnao et al.'s discolorizing process, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [5]. Samples and standard antioxidants (Trolox and α-tocopherol) were used in concentration ranges of 20-100 µg/mL. Using the following formula, the $ABTS^{\cdot+}$ radical scavenging activity was determined:

$ABTS^{\cdot+}$ radical scavenging activity (%) = $[(A_0\text{-}A_1)/A_0]$ x100

A_0 is the absorbance of the initial concentration of $ABTS^{\cdot+}$

A_1 is the absorbance of the remaining concentration of $ABTS^{\cdot+}$ in the presence of samples (or reference antioxidant).

2.2.2 $DPPH^{\cdot}$ radical scavenging activity assay

$DPPH^{\cdot}$ radical scavenging (radical quenching) activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) [6]. Sample and standard solutions (Trolox and α-tocopherol) were used in the concentration range of 20–100 µg/mL. The $DPPH^{\cdot}$ radical scavenging activity was calculated using the following formula:

$DPPH^{\cdot}$ radical scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where A_0 represents the initial absorbance of the $DPPH^{\cdot}$ solution, and A_1 represents the absorbance of the remaining $DPPH^{\cdot}$ in the presence of the samples (or reference antioxidant).

2.2.3 Cupric ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) assay

The total antioxidant capacity of the aqueous *C. coggygria* extract was determined using the Cu(II)-neocuproine reagent according to the method of Apak et al. [7]. Concentrations of ascorbic acid and BHT solutions in the range of 20–100 µg/mL were used as standards.

2.2.4 Reducing power assay

The reducing power of aqueous *C. coggygria* extract and reference samples (ascorbic acid and BHT) were performed according to the Oyaizu method [8].

2.2.5 Total phenolic contents (TPCs) assay

TPCs of the aqueous *C. coggygria* extract were obtained according to the Slinkard and Singleton method using the Folin-Ciocalteau reagent [9]. To get the calibration curve, pyrocatechin (20–100 µg/mL) solution was used as a reference. The TPCs in the aqueous *C. coggygria* extract were then measured in µg of pyrocatechin equivalent per milliliter of extract.

2.2.6 Total flavonoid contents (TFCs) assay

The total flavonoid contents (TFCs) of the aqueous *C. coggygria* extract were determined using the aluminum chloride colorimetric method according to Zhishen et al. [10]. Catechin solutions at concentrations ranging from 20 -100 µg/mL were used as standards. The TFCs of the plant extract was calculated and expressed as µg of catechin equivalents per milliliter of extract.

2.3 Pharmacological Study

2.3.1 Experimental animals and protocol

A total of 40 male Sprague–Dawley rats (6–8 months old, 220 ± 20 g) were housed in metal cages under a 12 h light/12 h dark cycle at room temperature under appropriate environmental conditions, with free access to food and water (*ad libitum*). The animals were obtained from the Department of Experimental Medical Research and Application (DETAE), Istanbul University, and were acclimatized for four weeks before the start of the experimental procedures. All experimental protocols were approved by the Istanbul University Animal Experiments Ethics Committee (Protocol No. 2015/68).

The animals were randomly assigned to four groups as follows: Group 1 (n = 8), control rats receiving 0.9% saline; Group 2 (n = 8), control rats receiving only *C. coggygria* aqueous extract (50 mg/kg); Group 3 (n = 10), experimental rats receiving 1 mL of absolute ethanol; and Group 4 (n = 14), rats receiving both *C. coggygria* aqueous

extract and absolute ethanol at the same doses via oral gavage. On the day of the experiment, the plant extract was administered 1 h before the administration of 1 mL of absolute ethanol (single-dose treatment). One hour after ethanol administration, the rats were anesthetized and euthanized. Brain tissues were collected and stored at −76°C until analysis.

All brain tissues were homogenized using glass homogenizers in physiological saline solution. The homogenates were centrifuged at 10,000 rpm for 15 min at +4°C. The resulting clear supernatants were transferred into Eppendorf tubes and stored at −76°C until further biochemical analyses.

2.3.2 Biochemical analyses

Brain tissue homogenates (10% w/v) were used for the determination of protein [11], reduced glutathione (GSH) [12], and lipid peroxidation (LPO) [13] levels, glutathione peroxidase (GPx) [14,15], glutathione reductase (GR) [16], glutathione-S-transferase (GST) [17], catalase (CAT) [18], superoxide dismutase (SOD) [19], acetylcholinesterase (AchE) [20] activities, nitric oxide (NO) [21] and sialic acid (SA) [22] levels, xanthine oxidase (XO) [23] activity, advanced oxidized protein products (AOPP) [24] content as well as aryl esterase (ARE) [25], carbonic anhydrase (CA) [26], paraoxonase (PON) [27], prolidase (PRO) [28], and lactate dehydrogenase (LDH) [29] activities and also reactive oxygen species (ROS) [30], total oxidant status (TOS) [31], total antioxidant status (TAS) [32].

2.3.3 *In vitro* effects of aqueous *C. coggygria* extract on ethanolic brain and pure LDH activity

The *in vitro* LDH inhibitory activity was determined and adjusted according to the Bais and Philcox method [33]. 160 µL $NADH^+$, H^+ (at a concentration of 170 µM), 5 µL enzyme source used either: ethanolic tissue LDH as %10 (w/v) homogenate (belonged to ethanolic group) or pure LDH enzyme as 0.1 U/mL. Aqueous *C. coggygria* extract was added to the reaction medium as an inhibitor in the concentration range of 5-40 mg/mL for ethanolic brain LDH. On the other hand, in pure LDH inhibition activity experiments, the concentration range was used as 0.25-5 mg/mL. After adding 20 µL of pyruvate solution (at a concentration of 14 mM), the reaction was initiated, and the absorbance change was observed spectrophotometrically at 340 nm at 37 °C. In addition to inhibitory activity calculations, IC_{50} values were also determined via regression analysis.

2.4 Statistical Analysis

GraphPad Prism 9.0 (GraphPad Software, San Diego, California, USA) was used to evaluate the biochemical data using Tukey's multiple comparison test, the unpaired t-test, and one-way analysis of variance (ANOVA). A value of $P<0.05$ considered statistically significant, and the findings were shown as mean ± standard deviation (SD).

3. Results

3.1 *In vitro* Antioxidant Activities

$ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical scavenging activities indicated as half-maximal inhibition concentrations (IC_{50}). IC_{50} is defined as the sample concentration required to achieve 50% free-radical neutralization; therefore, lower IC_{50} values indicate greater radical-scavenging activity. $ABTS^{\cdot+}$ radical scavenging activities of aqueous *C. coggygria* extract and reference substances are shown in Table 1. According to the results obtained, it was observed that aqueous *C. coggygria* extract exhibited very close IC_{50} value to the Trolox (IC_{50} = 69.759 ± 0.788 for *C. coggygria* extract; IC_{50} =66.752 ± 0.770 for Trolox, respectively). The order of $ABTS^{\cdot+}$ radical scavenging activities of plant extract samples and references IC_{50} values is as follows: Trolox < *C. coggygria* < α-tocopherol.

The $DPPH^{\cdot}$ radical scavenging activities of aqueous *C. coggygria* extract and inhibitors are presented in Table 1. When the obtained IC_{50} values were evaluated, it was seen that the plant extract had the lowest value compared to the references. The order of IC_{50} value of $DPPH^{\cdot}$ radical scavenging activities of aqueous *C. coggygria* extract and standards is as follows: *C. coggygria* < Trolox < α-tocopherol.

Table 1. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl, N,N-dimethyl-4- phenylenediamine radical scavenging activities of aqueous *C. coggygria* extract and standard samples.

	$ABTS^{\cdot+}$ IC_{50} (µg/mL)*	**$DPPH^{\cdot}$ IC_{50} (µg/mL)***
C. coggygria	69.759±0.788	1.340±0.493
α-Tocopherol[a,b]	154.198±78.139	14.085±0.653
Trolox[a,b]	66.752±0.770	2.645±0.302

*Mean±SD. ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), $DPPH^{\cdot}$: 2,2-Diphenyl-1-picrylhydrazyl, BHA: Butylated hydroxy anisole, a Standard for $ABTS^{\cdot}$+ radical scavenging activity, b Standard for $DPPH^{\cdot}$ and radical scavenging activity.

As shown in Table 2, all tested samples CUPRAC and reducing power values elevated with an increased concentration. At 100 µg/mL, extract and standards showed decreased CUPRAC levels as follows: BHT (1.626 ± 0.072) > *C. coggygria* extract (0.943 ± 0.066) > ascorbic acid (0.240 ± 0.020) (Table 2). On the other hand, when the reducing power values at 2000 µg/mL concentration were compared, the diminished values are in the order of BHT (0.301 ± 0.005) > *C. coggygria* extract (0.170 ± 0.001) > ascorbic acid (0.163 ± 0.004), respectively (Table 2). In both methods, *C. coggygria* had a high antioxidant activity compared to ascorbic acid. These findings indicate that the aqueous leaf extracts acted as effective electron and hydrogen donors, thereby interrupting radical chain reactions and converting free radicals into more stable products.

The TPCs and TFCs of aqueous *C. coggygria* extract are shown in Table 3. The TPCs increased with elevated concentrations and the TPCs values are ranging from 3.05 ± 0.05 to 8.18 ± 0.12 µg/g.

Table 2. The CUPRAC antioxidant capacities and reducing power levels of aqueous *C. coggygria* extract and standard samples.

	Conc. (µg/mL)	CUPRAC (Abs.)*	Conc. (µg/mL)	Red. Power (Abs.)*
C. coggygria	20	0.213±0.025	500	0.107±0.006
	40	0.421±0.008	1000	0.128±0.007
	60	0.629±0.020	1500	0.144±0.011
	80	0.799±0.005	2000	0.170±0.001
	100	0.943±0.066		
Ascorbic acid [a,b]	20	0.094±0.011	500	0.096±0.002
	40	0.157±0.027	1000	0.121±0.001
	60	0.197±0.030	1500	0.146±0.003
	80	0.211±0.024	2000	0.163±0.004
	100	0.240±0.020		
BHT [a,b]	20	0.394±0.010	500	0.201±0.007
	40	0.682±0.016	1000	0.233±0.008
	60	0.858±0.031	1500	0.257±0.005
	80	1.265±0.022	2000	0.301±0.005
	100	1.626±0.072		

*Mean±SD. CUPRAC: Cupric ions (Cu^{2+}) reducing antioxidant capacity, [a] Standard for CUPRAC, [b] Standard for reducing power.

The highest TPCs of extract was found at a concentration of 200 µg/mL (Table 3). On the other hand, TFCs values of the aqueous *C. coggygria* extract are ranging from 6.14 ± 0.20 to 19.20 ± 0.16 µg/g and the highest TPCs was found at a concentration of 2000 µg/mL (Table 3).

Table 3. Contents of total phenolics (TPCs) and total flavonoids (TFCs) of aqueous *C. coggygria* extract.

Extract Conc. (µg/mL)	TPCs (µg/g)*	Extract Conc. (µg/mL)	TFCs (µg/g)*
50	3.05±0.05	500	0.107±0.006
100	5.06±0.11	1000	0.128±0.007
150	6.44±0.18	1500	0.144±0.011
200	8.18±0.12	2000	0.170±0.001

*Mean±SD.

According to the results obtained, since *C. coggygria* has high antioxidant activity and total phenolic and flavonoid amounts, we examined its protective effect on brain damage induced by ethanol in our study.

3.2 *In vivo* Effects of Oral Administration of Aqueous *C. coggygria* Extract to Rats

As shown in Figure 2, there is a remarkable decline in the GSH level in the ethanol given animals compared to the non-treated animals (P<0.001). LPO level significantly increased in the ethanol given group (P<0.001) and *C. coggygria* aqueous extract administration reversed GSH and LPO values meaningfully according to the ethanol induced animals, respectively (P<0.001, P<0.0001).

GPx, GR, and GST activities (Figure 3) were decreased in ethanol induced animal brain tissues in comparison to control animals. While GPx activity has considerably decreased (P<0.01), this decrease is insignificant in the activities of GR and GST. Administration of the *C. coggygria* aqueous extract increased those levels meaningfully as to ethanol given rats (P<0.0001 for each parameter). CAT and SOD activities (Figure 3) diminished in ethanol given group and *C. coggygria* extract reversed these values meaningfully (P<0.001 for each parameter)

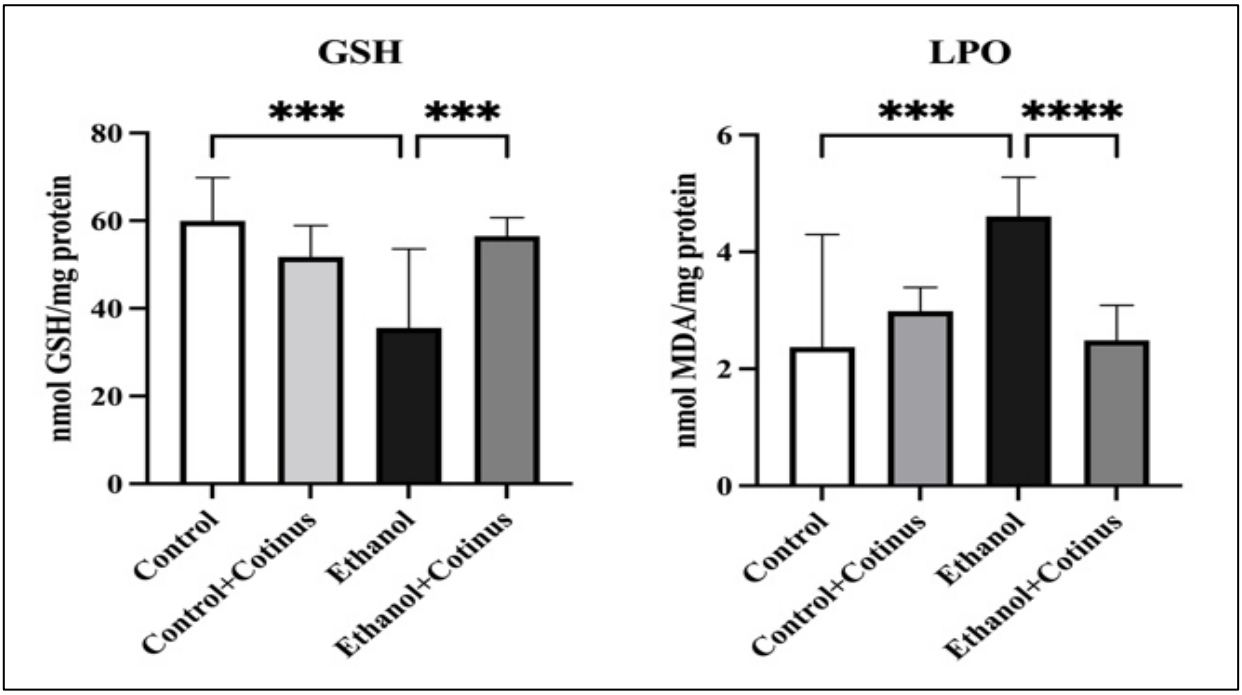


Figure 2. The brain (A) reduced glutathione (GSH) and (B) lipid peroxidation (LPO) levels of all groups (****P < 0.0001, ***P < 0.001).

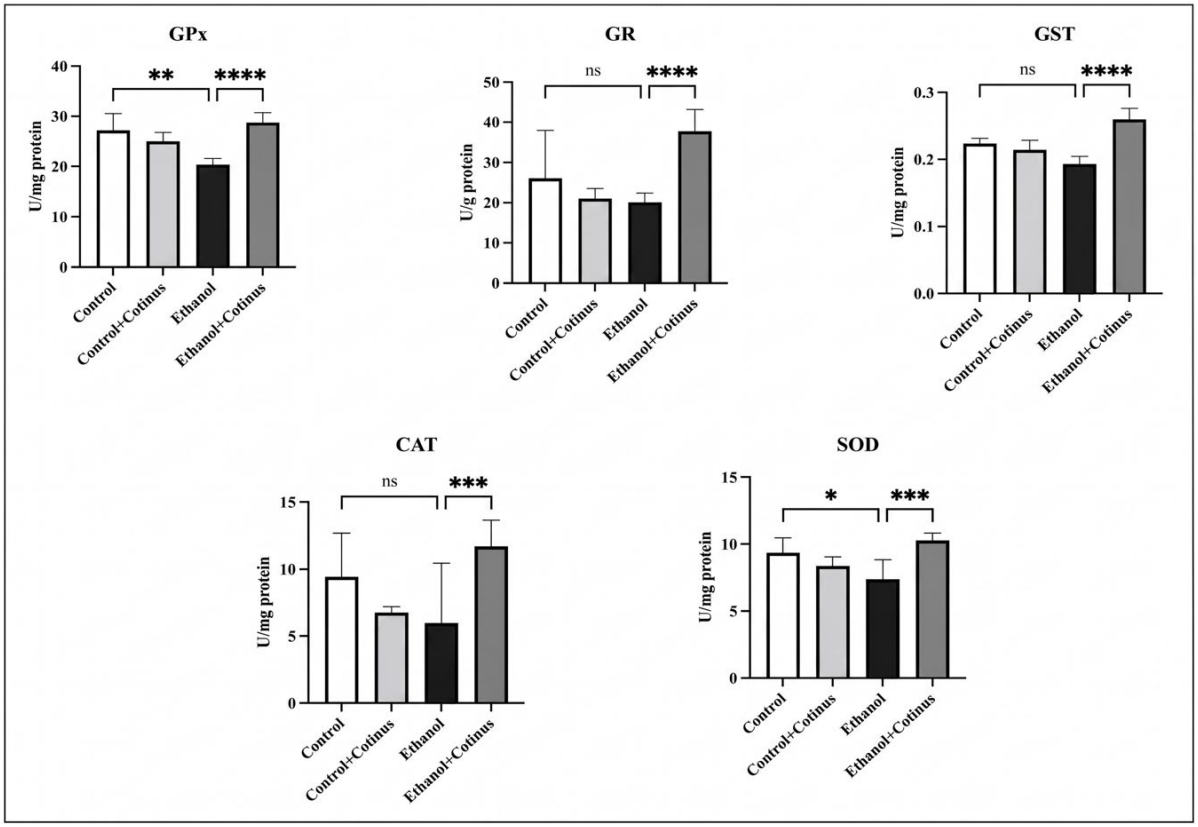


Figure 3. The brain glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) activities of all groups ($^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns $P > 0.05$).

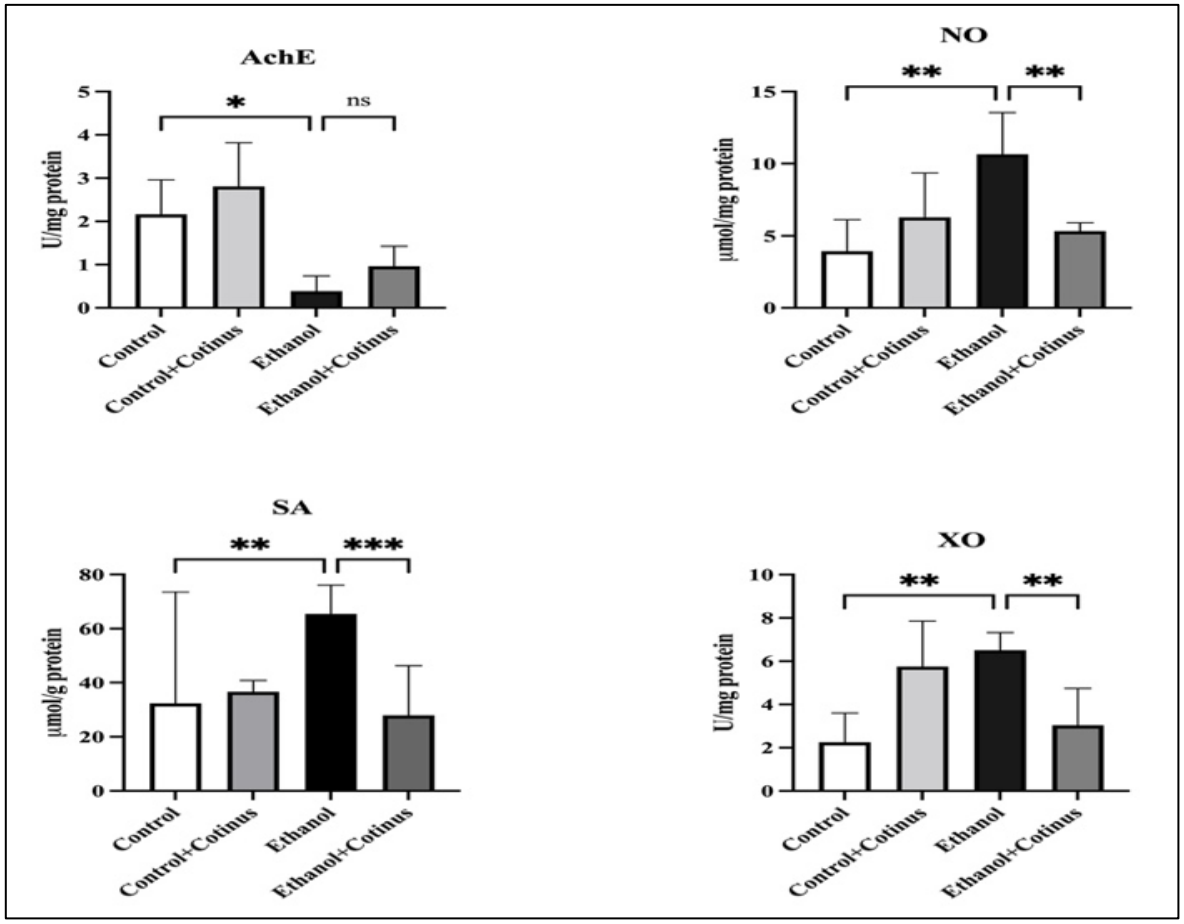


Figure 4. The brain acetylcholinesterase (AChE) activity, nitric oxide (NO) and sialic acid (SA) levels, xanthine oxidase (XO) activity of all groups ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns $P > 0.05$).

The activity of AChE (Figure 4) indicated a considerable reduction in ethanol given animals compared to the control group (P<0.05). Ethanol elevated NO and SA levels (Figure 4) in brain tissues (P<0.01 for each parameter). Plant extract administration notably decreased these values when compared with ethanol given rats, respectively (P<0.01, P<0.001). XO activity (Figure 4) increased in the ethanol given animals in contrast to the non-treated group (P<0.01) and aqueous plant extract administration reversed these values (P<0.01).

Experimental groups are presented in Figure 5, AOPP level was elevated significantly compared to control group (P<0.0001) and AOPP level diminished in *C. coggygria* treated group (P<0.0001).

ARE, CA, and PON activities (Figure 5) increased in the plant extract treated animals in comparison to the ethanol given animals, respectively (P<0.001, P<0.0001, P<0.01). The activity of PRO (Figure 5) demonstrated a considerable increase in ethanol given animals in comparison to control group (P<0.0001). In comparison to rats administered ethanol, aqueous plant extract treatment significantly reduced these levels (P<0.001).

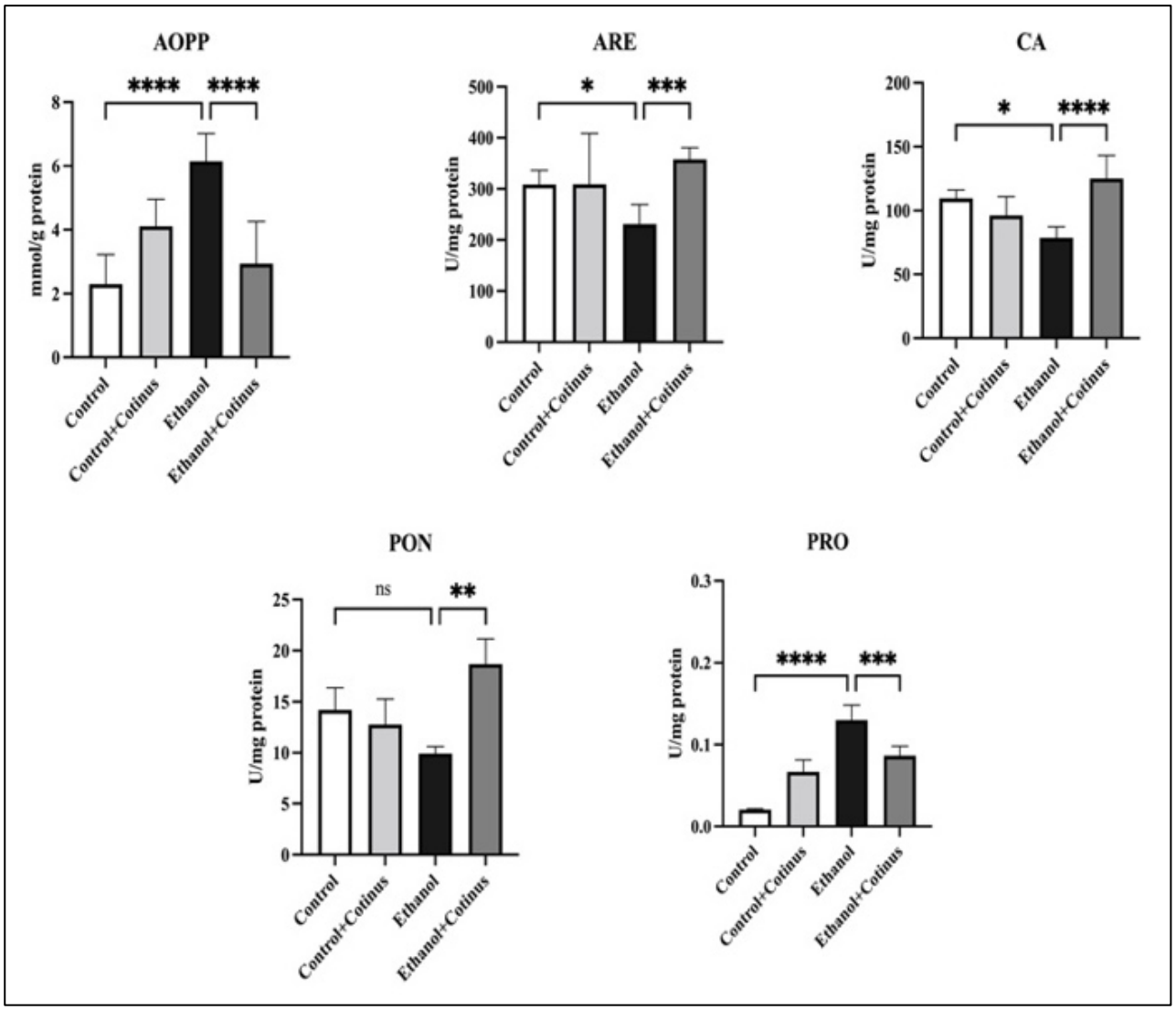


Figure 5. The brain advanced oxidized protein product (AOPP) level and aryl esterase (ARE), carbonic anhydrase (CA), paraoxonase (PON) and prolidase (PRO) activities of all groups ($^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ns $P > 0.05$).

In the ethanol given animals, LDH, ROS and TOS levels (Figure 6) notably increased, respectively (P<0.0001, P<0.01) whereas TAS level (Figure 6) diminished in comparison to the non-treated group (P<0.001). *C. coggygria* aqueous extract administration reversed these values meaningfully according to the ethanol induced animals, respectively (P<0.0001, P<0.01).

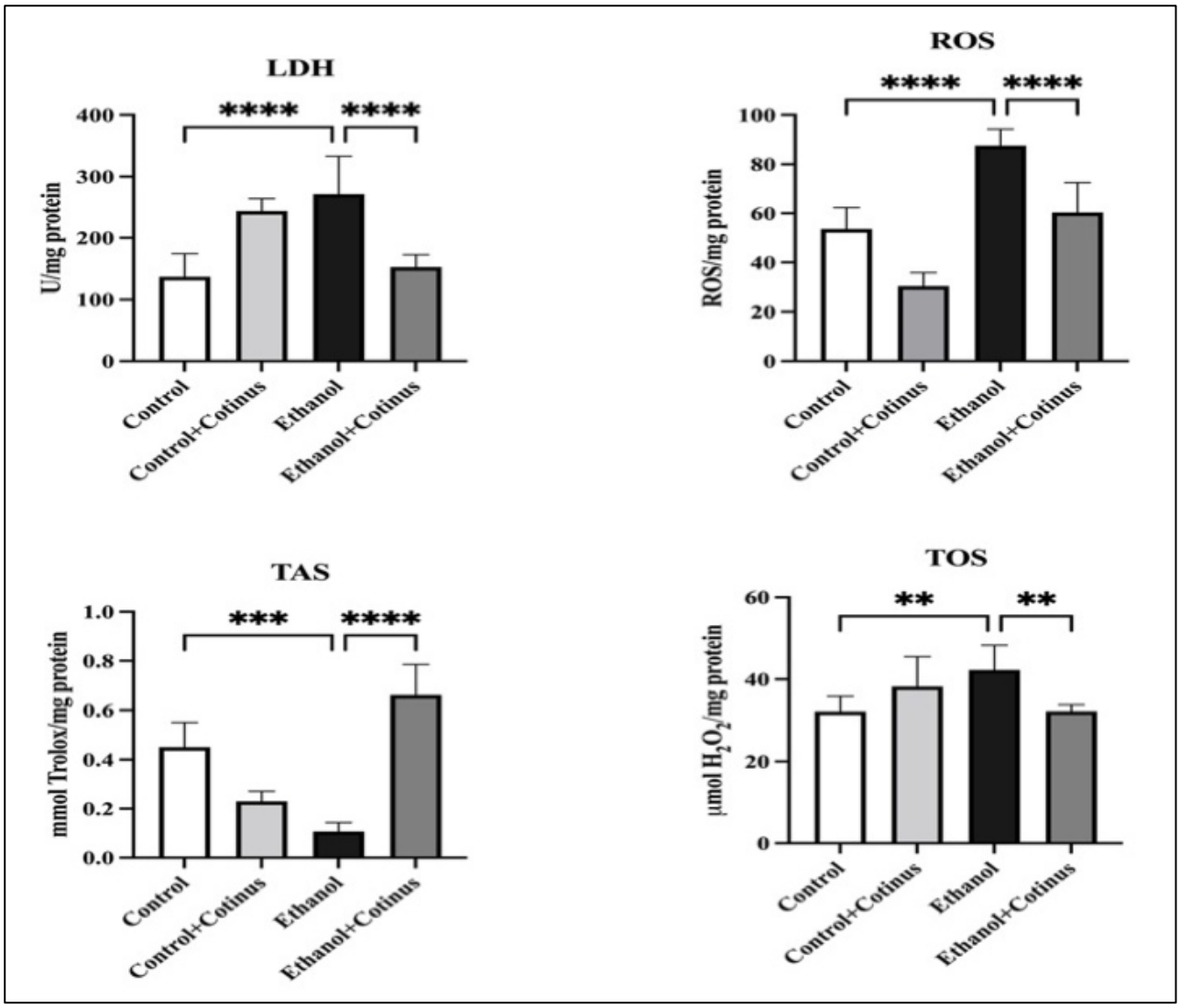
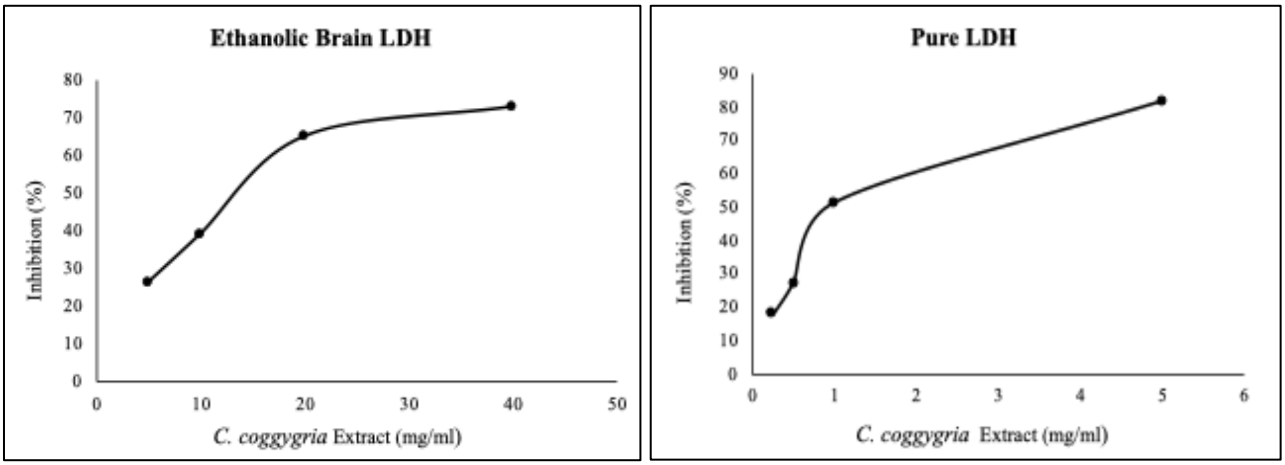


Figure 6. The brain lactate dehydrogenase (LDH) activity, reactive oxygen species (ROS) and total antioxidant status (TAS), total oxidant status (TOS) activity of all groups ($^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$).

3.3 *In Vitro* Effects of Aqueous *C. coggygria* and Ethanol on LDH Activity

In vitro inhibition of aqueous *C. coggygria* extract on ethanolic brain tissue LDH enzyme and pure LDH enzyme are given in Figure 7. According to the obtained results *C. coggygria* inhibited increased LDH activity in brain tissue given ethanol with IC_{50}= 18.80 ± 3.16 at 5-40 mg/mL range. The pure LDH is inhibited by extract with IC_{50}= 2.10 ± 0.34 at 0.25-5.0 mg/mL.



A B

Figure 7. The *in vitro* inhibitor effects of aqueous *C. coggygria* extract on brain tissue LDH enzyme (A) and pure LDH enzyme (B) activities.

The secondary metabolites in plants have pharmacological effects such as antioxidant and anti-inflammatory [34,35]. Hydrolysable gallotannins, particularly pentagalloyl-O-glucose, have been identified as contributors to the pharmacological effects of *C. coggygria* extracts. These components were observed to block enzymes, such as prolyl oligopeptidase, which is linked to the formation and growth of tumors. The chemical composition and antioxidant potential in the different parts and developmental processes of *C. coggygria* emphasized the abundant flavonoids phenolic acids (gallic acid, chlorogenic acid, caffeic acid and rosmaric acid), flavonoids (sulfuretin, cotinignan A, butein, taxifolin, fisetin, myricetin, kaempferol, apigenin, petunidin-3-glucoside, delphinidin-3-galactoside and cyanidin-3-galactoside) hydrolysable tannins derived from gallic acid and terpenes [36]. In the current research, various spectrophotometric methods have been used to characterize the general antioxidant status in *C. coggygria* aqueous extract. The obtained results demonstrated that the *C. coggygria* aqueous extract exhibited high total phenolic and flavonoid contents, along with strong $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radical scavenging activities. Moreover, according to the values obtained using the CUPRAC method, which is based on the reliable determination of total polyphenols, as well as vitamins C and E, it is observed that the aqueous *C. coggygria* extract has a significantly high antioxidant capacity. Similarly, to our findings in the study investigating the antioxidant content of *C. coggygria* leaves, it was reported that this part of the plant has a high phenolic (327.33 ± 3.28 mg/g extract) and flavonoid (48.20 ± 2.15 mg/g extract) contents [37].

Ethanol causes the free release of superoxide radicals in the presence of oxygen by the cytochrome P450 superfamily (especially CYP2E1) in both adults and fetuses, inducing the oxidation of polyunsaturated fatty acids to toxic aldehyde products. During the metabolism of alcohol in the liver, free radicals are produced, and GSH helps control lipoperoxide formation by oxidizing GSH to glutathione disulfide (GSSG) in tandem with GPx to neutralize these free radicals [38]. Another significant factor that leads to the harmful effects of ethanol is highly reactive lipid aldehydes such as malondialdehyde (MDA). This process results from triggering the production of free radicals and/or establishing acetaldehyde are integrally related to harm caused in the brain and various other organs [39]. In alignment with previous research, our study also highlights an increased LPO level in brain tissue exposed to ethanol, in comparison to the intact group, and the levels of reduced glutathione were found to be decreased. These alterations were accompanied by a reduction in the activities of the antioxidant enzymes GPx, GR, GST, SOD, and CAT in the ethanol-exposed group. Due to the low antioxidant capacity of neurons and their richness in PUFA components, GPx exhibits high activity in the central nervous system, making the tissue highly susceptible to oxidative stress [40]. GR, which is abundantly present in neurons and glial cells, has been reported to be highly sensitive in the cerebral cortex after acute ethanol exposure [41]. SOD acts to convert the superoxide anion into H_2O_2, subsequently broken down by CAT. Accordingly, reduced SOD and CAT activities are often interpreted as an adaptive response to oxidative stress. In our study, it was clearly observed that oral administration of *C. coggygria* extract significantly increased the antioxidant activity of these enzymes.

In this study, a single exposure to ethanol reduced AChE activity. In contrast, another study showed that both acute and chronic ethanol consumption increased blood NO levels in animals and humans [42]. The alterations induced by ethanol on NO production and neuronal nitric oxide synthase NOS activity may be crucial in modifying the function of cells or organs affected by ethanol. Sogut and Kanbak (2010) shows that exposure of rat brain synaptosomes *in vitro* to varied doses of ethanol (50 mM, 100 mM and 200 mM) resulted in an increase in SA levels [43]. On the other hand, it has been observed that ethanol increases hypoxanthine and xanthine levels in liver tissue, thereby also leading to an increase in allantoin. Additionally, it has been revealed that ethanol causes an increase in NADH levels in the same organ and subsequently reduces xanthine dehydrogenase activity. Increased purine degradation, presumably related to a shift from the dehydrogenase to the XO pathway, is hypothesized as a possible mechanism for ethanol-induced generation of free radicals [44]. In this study, decreased AChE activity, as well as increased XO activity, NO and SA levels, were found in the brain tissue of rats exposed to ethanol. Treatment with *C. coggygria* reversed/diminished these activities and levels in the ethanolic group. It is possible that the high phenolic and flavonoid content of *C. coggygria* contributed to this effect.

AOPP is a marker used to detect oxidant-mediated protein damage and consequently estimate its severity. On the other hand, high levels of proline and PRO activity are associated with cognitive impairments, intellectual disabilities, Schizophrenia, Parkinson's disease, Alzheimer's disease, and brain damage caused by reduced blood flow [45]. In liver tissue, it has been reported that excessive alcohol consumption can lead to a decrease in PON1 and ARE activities [46]. Supporting these data, we also observed a considerable elevation in AOPP and PRO and decrease in PON and ARE activities as a result of acute ethanol exposure in comparison to the control group. Besides, CA which has synthesis and activating effects towards four human CA isoforms expressed in the human brain, also shows decreased activity in the ethanol exposure. It was observed that the application of the plant extract to ethanol-treated groups reversed these values and, consequently, had a positive and protective effect on both enzymatic and non-enzymatic antioxidant defense systems.

Both biochemical and histopathological examinations confirmed that increased LDH activity in the hippocampus was due to increased ROS resulting from chronic ethanol exposure [47]. In support of this finding, we also observed a significantly increased activity in LDH in ethanolic brain

tissue. Besides that, we observed that *C. coggygria* extract administered to ethanolic rats also successfully reduced *in vitro* LDH activity both in ethanolic brain tissue and pure LDH.

Considering the ROS, TAS, and TOS parameters, the final findings of our study, it was observed that all three parameters were significantly elevated in the ethanol-treated group compared to the control group. This suggests that ethanol shifts the oxidant/antioxidant balance in brain tissue in favor of oxidants. It was observed that the application of the plant extract significantly reversed these values. This finding can be attributed to the plant's high antioxidant content, which provides effective protection to brain tissue by neutralizing free radicals.

4. Conclusion

In summary, alcohol use disorder is a very common health problem, so protecting the brain from damage caused by ethanol byproducts is of critical importance. The presented study shows in detail the effects of *C. coggygria* aqueous extracts in preventing ethanol-induced oxidative stress in rat brain tissues due to their antioxidant capacity. According to the results, it can be suggested that the antioxidant compounds found in *C. coggygria* extract have a therapeutic effect on ethanol-induced brain oxidative stress parameters in the ethanol-induced rat model. We think that these promising results may be useful for researchers interested in this field.

Conflict of Interest

There are no conflicts of interest in this work.

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Ethical Approval Statement

This study was approved by the Istanbul University-Animal Experiments Ethics Committee (Approval no: 2015/68).

Declaration of Generative AI

The authors confirm that no generative AI technologies were used in the preparation, writing, or data analysis of this manuscript.

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