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KEÇİBOYNUZUNUN (CERATONİA SİLİQUA L.) NİKOTİN BAZLI OKSİDATİF STRESE ETKİSİ

THE EFFECT OF CAROB (CERATONIA SILIQUA L.) AGAINST NICOTINE BASED OXIDATIVE STRESS

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ÖZET

AMAÇ: Bu çalışmada keçiboynuzu bitkisinin antioksidan etkisi, nikotin kaynaklı oksidatif strese ve rat hipokampüsü cornu ammonisinde (CA) piramidal hücre sayısına etkisi araştırılmıştır.

GEREÇ VE YÖNTEM: Çalışmada 28 adet yetişkin Wistar Albino erkek rat kullanıldı. Ratlar kontrol grubu, keçiboynuzu grubu, nikotin grubu ve nikotin+keçiboynuzu grubu olmak üzere dört gruba ayrıldı. Deney grubuna nikotin, tedavi grubuna ise nikotin ile birlikte keçiboynuzu ekstresi uygulandı. Akciğer, beyin, böbrek, kalp ve karaciğer dokularında superoksit dismutaz (SOD), glutatyon (GSH), glutatyon disülfit (GSSG), total oksidan seviye (TOS), total antioksidan seviye (TAS) değerleri spektrofotometrik analiz ile ölçüldü. Oksidatif stres indeksi (OSI) ve GSH/GSSG değerleri sırasıyla TOS/TAS ve GSH/GSSG oranları şeklinde hesaplandı. CA'daki piramidal hücre sayısı, optik fraksiyonlama tekniği kullanılarak belirlendi.

BULGULAR: Nikotin grubunun böbrek dokusundaki TAS düzeyinin kontrol ve keçiboynuzu gruplarına göre anlamlı derecede düşük olduğu görüldü. Beyin dokusunda, nikotin grubunun TAS düzeyi diğer gruplara göre önemli ölçüde düşüktü ($p<0.001$). Nikotin grubunun OSI değeri karaciğer dokusunda kontrol grubuna göre anlamlı derecede yüksekti ($p<0.001$). Nikotine maruz kalmanın CA'daki piramidal hücre sayısında önemli bir azalmaya neden olduğu gösterildi.

SONUÇ: Keçiboynuzu bitkisinin oksidatif stres oluşumunda TAS düzeyini artırarak nikotine karşı antioksidan özelliklere sahip önemli bir fitomedikal bitki olabileceği kanısına varılmıştır.

ANAHTAR KELİMELER: Keçiboynuzu özü, Nikotin, Oksidatif stres, Piramidal hücreler.

ABSTRACT

OBJECTIVE: In this study the antioxidant effect of carob plant, ıt's effects of nicotine-induced oxidative stress and the number of pyramidal cells in the rat hippocampus in cornu ammonis (CA) were investigated.

MATERIAL AND METHODS: In the study, 28 adult Wistar Albino male rats were used. Rats are divided into four groups as control group, carob group, nicotine group and nicotine + carob group. Nicotine was applied to the experimental group, and carob extract was applied to the treatment group as well as nicotine. Superoxide dismutase (SOD), glutathione (GSH), glutathione disulfide (GSSG), total oxidant capacity (TOS), total antioxidant capacity (TAS) values were measured by spectrophotometric analysis on the lung, brain, kidney, heart and liver tissues. Oxidative stress index (OSI) and GSH / GSSG values, respectively, were calculated as TOS/TAS and GSH/GSSG rates. The number of pyramidal cells in the CA was estimated using the optical fractionator technique.

RESULTS: It is seen that the TAS level in the kidney tissue of the nicotine group is significantly lower than the control and carob groups. In brain tissue, the TAS level of the nicotine group was significantly lower than that of other groups ($p<0.001$). The OSI value of the nicotine group was significantly higher in liver tissue compared to the control group ($p<0.001$). Nicotine exposure has been shown to cause a significant reduction in the number of pyramidal cells in CA.

CONCLUSIONS: It is understood that Carob plant is an important phytomedical plant that has antioxidant properties against nicotine by increasing TAS level in oxidative stress formation.

KEYWORDS: Carob extract, Nicotine, Oxidative stress, Pyramidal cells.

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INTRODUCTION

Smoking and exposure to nicotine are prevalent social habits that pose a major harm. In fact, due to the realization that they are safer than conventional tobacco use, electronic nicotine delivery systems (ENDS) or electronic cigarettes (E-Cig) that have recently appeared have greatly increased in popularity, especially among young populations (1). It is known that the electronic cigarettes used also have nicotine-containing forms (2). The point we want to draw attention to here is the nicotine substance found in tobacco products consumed. Nicotine is the primary ingredient that causes addiction in tobacco products. Nicotine is an alkaloid compound extracted from the leaves of the tobacco plant. Nicotine, one of the main toxic components of cigarette smoke, can be rapidly absorbed by the respiratory system and reach high levels in the blood and brain (3 – 6). Negative effects on immunological, genetic, and reproductive systems have also been shown, in addition to their role in the origins of cancer and cardiovascular diseases. The balance between oxidant and antioxidant systems is held responsible for the occurrence of these negative effects (4). By breaking the mitochondrial respiratory chain and promoting the generation of reactive oxygen species (ROS), including hydrogen peroxide, nicotine is known to cause oxidative stress (3, 7).

Oxidative stress occurs as a result of the disturbance between free radicals resulting from cellular events and antioxidants (8, 9). Free radicals here; they can be endogenous (as a result of oxygen use during aerobic metabolism) or exogenous (with the effect of smoking, air pollution, sun rays, X-Ray) (8,10). There are defense mechanisms called antioxidants in order to prevent oxidative stress caused by free radicals, especially ROS, and related tissue damage. Medicinal and aromatic plants with rich phenolic content are also used as antioxidant sources (9, 11). Carob is also an antioxidant plant (12). In the world, carob mostly has a Mediterranean climate; Spain, Italy, Morocco, Portugal, Greece, Cyprus and Turkey are grown in countries like (13). The antioxidative properties of carob extract, rich in phenolic substances, have been demonstrated by different studies (14, 15). Some of the studies focused on the hippocampus, a part of the brain that controls crucial behavioral and cognitive processes, such as mechanical learning and labor (16,17). There are studies indicating that nicotine crosses the blood brain barrier and prepares the ground for functional loss in the brain (18,19).

In our study, we evaluated the antioxidative effect of carob plant against nicotine-induced oxidative stress in terms of biochemical parameters and pyramidal cells.

MATERIALS AND METHODS

Study Design and Animals Groups

The study used 28 Wistar Albino rats (adult male) weighing 180–220 g. One week prior to the experiment, the animals had unrestricted access to food and water to ensure their comfort in the lab. Animal welfare requirements called for frequent cleaning and ventilation of cages. Throughout the experiment, the animals were kept in a temperature-controlled environment (20–23 ^{o}C) with a 12-hour light/dark cycle.

Experimental Groups

During the experiment, 0.9 percent saline was injected subcutaneously (s.c.) once daily into the control group (C) (n=6). 800mg/kg of carob extract was administered by gavage to the carob group (CRB) (n=7). Nicotine group (N), n=7, received 4 mg/kg of nicotine (s.c)). The nicotine + carob (N + CRB) group (n = 8) received 4 mg/kg of nicotine and 800 mg/kg of carob extract. All groups had these procedures for 35 days.

Preparation of Nicotine and Carob Extract

Nicotine hydrogen tartrate (95% nicotine: Sigma, USA) was used in the study. As the solvent solution, normal saline was used to adjust the amount of nicotine to be given to animals. 100 g of dried carob powder was mixed with 1000 ml distilled water. The mixture was made for 24 hours at room temperature. This mixture was passed through a Whatman No. 1 filter, and evaporated at 40°C water and concentrated. This extract was dissolved in saline and injected intraperitoneally (i.p.).

Taking Tissue Samples

At the conclusion of the 35-day trial period, all animals were sacrificed using the vertebral cervical dislocation technique while anesthetized

with xylazine/ketamine (75 mg/kg)-(10 mg/kg) in accordance with the procedure suggested by the Institutional Animal Ethics Committee. The organ tissues (liver, kidney, heart, lung, and brain) had the connective tissue around them removed and kept for the experiment.

Antioxidant indices and cytokines measurements

Using modified techniques, the activities of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione disulfide (GSSG), and glutathione peroxidase (GPx) were evaluated in blood and tissue samples (20). To test the levels of total oxidative status (TOS; Rel Assay Diagnostics, Gaziantep Turkey), (21), total anti-oxidative status (TAS, Rel Assay Diagnostics, Gaziantep Turkey) (22), and thiobarbituric acid reactive substances/malondialdehyde (TBARS/MDA; E-BC-K025-S, Elabscince, Houston, US) (23), colorimetric kits were used. Analyses of each sample were performed twice. Using the equation GSH = T-GSH - (2 x GSSG), the glutathione content was determined. The formula GSH = GSHt – 2 x GSSG was used to determine the levels of GSH. The values for GSHt, GSH, and GSSG were converted to nmol of GSH or GSSG per mg of protein after being normalized to total protein content (nmol GSH/mg protein or nmol GSSG/mg protein). The formula used to determine the oxidative stress index (OSI) was OSI = [TOS (mol H2O2 equiv./l) / TAS (mol trolox equiv./l) x 100]. Commercial ELISA assay kits were used to evaluate the cytokines serum levels (IL-6, TNF) (Elabscience, MD, USA).

Stereological Analysis System and Stereological Analyses

Light Microscope Procedure

Brain hemispheres were routinely kept in 10% formaldehyde solution for histopathological and stereological analysis. After the tissues were embedded in paraffin blocks, 20 µm thick sections were taken in the coronal plane. After the taken tissues were placed in slides with polylysine, they were kept in incubator for one night. The next day, it was examined under the light microscope for histopathological evaluation of the tissues taken from the incubator.

Stereological Procedure

Using the optical disector technique, the number of pyramidal cells in the CA region of the hippocampus was calculated (17, 24, 25). The sections were chosen for the calculations using a systematic random sampling method. Cross-section sampling rate was used to determine the number of pyramidal neurons in the cornu ammonis (CA) region of the hippocampus.

For stereological analyses, we employed a stereology workstation in Kayseri, Turkey. A CCD digital camera, an image capture card, a computer, a motorized specimen stage controlled by a computer (Prior Scientific, Cambridge, UK), a microcator (Heidenhein Traunreut, Germany), and a light microscope made up this setup (Leica, Nubloch, Germany). To measure, record, and take digital pictures of the sections, we used a software program called Macrobrightfield. To count the total number of Pyramidal cells in the hippocampus CA regions, the system generated microscope images [a 100 x Leica HCX Plan Apo objective] and presented them on a computer screen.

The hippocampus's CA region's total number of pyramidal neurons (N) was determined using the equation $N = \Sigma Q . \frac{1}{ssf} . \frac{1}{asf} . \frac{1}{tsf}$

N stands for total neurons, ΣQ for total disector neurons, ssf for sectional sampling, asf for area sampling, and tsf for thickness sampling. In conclusion, the Pyramidal cell was counted if the largest nuclear profile was seen within the unbiased virtual counting frames spread randomly and methodically throughout the designated regions. The total number of Pyramidal cells was determined based on the counted number of Pyramidal cells and the sample probability (26, 27). The optical dissector method was used to determine how many pyramidal neurons were present in the CA region of the hippocampus.

Ethical Committee

Rats were handled in accord with institutional policies and the National Research Council's Guide for the Care and Use of Laboratory Animals. The ethics committee approval numbered 2019/31 from the Erciyes University Animal Experiments Local Ethics Committee in 2019.

Statistical Analysis

For statistical analysis, the SPSS 22 package program was used. The results are presented as mean ± standard deviation. One-way

ANOVA was used to compare groups. The difference between the groups was determined using the post-hoc Tukey test. A p value of 0.05 or above was considered important statistically. Pyramidal cells in the hippocampus CA region were compared using the Kruskal Wallis test. For each group, the data were presented as mean ± standard deviation.

RESULTS

The tables below present the results of biochemical analysis on antioxidant enzymes and oxidative stress parameters for various groups in rat lung, brain, kidney, heart, and liver tissues **(Table 1-5).** The markers of oxidative stress in lung tissue, TBARS, TOS, and OSI values, were found to be significantly higher in the N group than in the C group (p<0.001). It was seen that TAS, which is an indicator of antioxidant capacity, is in the highest CRB group. In addition, it was concluded that TAS value has close values in C and CRB groups (p>0.05). When the enzyme activities were evaluated, it was found that the group with the highest SOD and GPx activities was the N group. CAT activity was found to be close to each other in the C and N groups (p>0.05) and significantly higher than other groups (CRB and N+CRB) (p<0.001) **(Table 1)**.

Table 1: Biochemical analysis results on oxidative stress parameters and antioxidant enzymes in lung tissue by groups

Parameters\Groups	C	CRB	N	N+CRB	*p*
TBARS	4.03±0.19[ac]	3.51±0.26[a]	5.02±0.58[b]	4.18±0.31[c]	<0.001
SOD	9.08±0.39[a]	8.86±0.25[a]	13.43±0.34[b]	11.56±0.31[c]	<0.001
CAT	52.9±3.47[a]	46.58±2.25[b]	52.15±1.6[a]	46.98±3.22[b]	<0.001
GPx	6.55±0.16[a]	6.29±0.11[a]	10.11±0.43[b]	8.79±0.55[c]	<0.001
TAS	0.95±0.03[a]	0.97±0.07[a]	0.57±0.02[b]	0.64±0.01[b]	<0.001
TOS	3.82±0.09[a]	4.11±0.28[a]	5.27±0.96[b]	4.17±0.13[a]	<0.001
OSI	0.4±0.01[a]	0.42±0.03[a]	0.92±0.18[b]	0.65±0.03[c]	<0.001
GSH	5.68±0.13[a]	5.74±0.1[a]	4.48±0.49[b]	5.36±0.08[a]	<0.001
GSSG	0.87±0.05[a]	0.84±0.02[a]	1.52±0.24[b]	1.32±0.08[c]	<0.001
GSH/GSSG	4.5±0.26[a]	4.78±0.12[a]	1.02±0.72[b]	2.05±0.25[c]	<0.001
Redox potential	-71.01±0.28[a]	-71.71±0.31[a]	-57.66±3.76[b]	-64.1±0.82[c]	<0.001

Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different (p < 0.001). C, *control*; CRB, *carob*; N, *nicotine*; N+CRB, *nicotine+carob*; TBARS, *thiobarbituric acid reactive substance;* SOD, *superoxide dismutase;* CAT, *catalase;* GPx, *glutathione peroxidase;* TAS, *total antioxidative status;* TOS, *total oxidative status;* OSI, *oxidative stress index;* GSH, *glutathione;* GSSG, *glutathione disulfide*

Table 2 shows the results of biochemical analysis on brain tissue. When TOS and OSI values, which are markers of oxidative stress, are examined, it is seen that these values are significantly higher in N group compared to other groups (p<0.001). Although the TBARS value has the highest value in the N group, it is seen that this value is close to each other and the difference between them is meaningless (p>0.001). The group with the TAS value at the lowest level was the N group (p<0.001). When the TAS value of other groups is examined, it is seen that they are close to each other (p>0.05). When SOD activities are examined, it is understood that there is no significant difference between the groups and the values are close to each other (p>0.001). CAT activity was calculated at close values (p>0.05) in C and CRB groups and at significantly higher value from other groups (N and N+CRB) (p <0.001). It is observed that GPx activity, which is in the close values (p>0.05) in the N and N+CRB groups, is higher than the other groups (C and CRB) (p<0.001), (Table 2).

Table 2: Biochemical analysis results on oxidative stress parameters and antioxidant enzymes in brain tissue by groups

Parameters\Groups	C	CRB	N	N+CRB	*p*
TBARS	3.36±0.17[a]	3.55±0.31[ab]	3.88±0.11[b]	3.59±0.32[ab]	0.011
SOD	13.84±0.83[a]	12.78±0.44[b]	12.61±0.86[b]	13.29±0.66[ab]	0.018
CAT	7.88±0.87[a]	6.96±0.38[a]	3.79±0.86[b]	5.71±0.8[c]	<0.001
GPx	25.34±1.73[a]	24.72±1.18[a]	31.45±1.34[b]	31.57±1.37[b]	<0.001
TAS	1.02±0.05[a]	1.05±0.08[a]	0.49±0.03[b]	0.99±0.12[a]	<0.001
TOS	7.19±0.92[a]	7.15±0.63[a]	11.49±1[b]	8.08±0.42[a]	<0.001
OSI	0.7±0.11[a]	0.68±0.1[a]	2.34±0.3[b]	0.82±0.11[a]	<0.001
GSH	6.68±1.59[a]	5.76±1.67[ab]	4.01±0.72[b]	5.78±0.46[ab]	0.005
GSSG	0.4±0.04[a]	0.4±0.03[a]	0.61±0.03[b]	0.47±0.08[a]	<0.001
GSH/GSSG	14.64±4.71[a]	12.53±4.58[a]	4.57±1.29[b]	10.52±2.16[a]	<0.001
Redox potential	-84.56±7.31[a]	-80.73±7.32[a]	-66.27±4.78[b]	-79.58±3.06[a]	<0.001

Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different (p<0.001). C, *control*; CRB, *carob*; N, *nicotine*; N+CRB, *nicotine+carob*; TBARS, *thiobarbituric acid reactive substance;* SOD, *superoxide dismutase;* CAT, *catalase;* GPx, *glutathione peroxidase;* TAS, *total antioxidative status;* TOS, *total oxidative status;* OSI, *oxidative stress index;* GSH, *glutathione;* GSSG, *glutathione disulfide*

Biochemical analysis results in kidney tissue are shown in Table 3. Looking at the TBARS level, it is seen that it is the lowest value in the C group. The group with the lowest SOD activity is the N + CRB group; CAT and GPx activities were found to be significant in the C and CRB groups according to statistical results (p<0.001). TAS level is close to each other in C and CRB groups (p>005), and it is significantly higher than N applied groups (p<0.001). It is seen that TOS and OSI levels are close in the N and N+CRB groups (p>0.05) and these values are significantly lower in the other groups (C and CRB groups) (p<0.001) **(Table 3)**.

Table 3: Biochemical analysis results on oxidative stress parameters and antioxidant enzymes in kidney tissue by groups

Parameters\ Groups	C	CRB	N	N+CRB	*p*
TBARS	2.91±0.26[a]	3.28±0.25[ab]	3.62±0.25[b]	3.23±0.3[ab]	<0.001
SOD	22.26±0.2[a]	20.32±0.79[b]	21.87±1.58[a]	18.62±0.87[c]	<0.001
CAT	23.82±0.46[a]	20.93±1.1[a]	33.91±5.31[b]	32.96±5.83[b]	<0.001
GPx	70.26±2.53[a]	67.82±1.06[a]	87.91±9.72[b]	80.5±1.39[b]	<0.001
TAS	1.38±0.05[a]	1.43±0.12[a]	1.1±0.15[b]	1.17±0.14[b]	<0.001
TOS	0.57±0.06[a]	0.57±0.03[a]	0.69±0.11[b]	0.79±0.68[b]	<0.001
OSI	0.04±0[a]	0.04±0[a]	0.06±0.01[b]	0.06±0.01[b]	<0.001
GSH	5.14±0.99[a]	4.3±1.1[a]	4.36±0.74[a]	4.59±0.36[a]	0.297
GSSG	1.64±0.15[ab]	1.46±0.11[b]	1.79±0.29[a]	1.73±0.13[a]	0.011
GSH/GSSG	1.15±0.78[a]	0.94±0.72[a]	0.45±0.38[a]	0.64±0.04[a]	0.137
Redox potential	-59.77±5.88[a]	-56.41±6.26[a]	-54.58±3.44[a]	-56.53±1.05[a]	0.281

Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different (p < 0.05). C, *control*; CRB, *carob*; N, *nicotine*; N+CRB, *nicotine+carob*; TBARS, *thiobarbituric acid reactive substance;* SOD, *superoxide dismutase;* CAT, *catalase;* GPx, *glutathione peroxidase;* TAS, *total antioxidative status;* TOS, *total oxidative status;* OSI, *oxidative stress index;* GSH, *glutathione;* GSSG, *glutathione disulfide*

The values shown in Table 4 belong to the results of biochemical analysis in the heart tissue. It is seen that TBARS level is significantly lower in group C compared to other groups (p<0.001). SOD activity was calculated at the highest value in the N group. It is observed that CAT and GPx activities are at the lowest level in the CRB group and these values in the C group are close to the values in the CRB group (p>0.05). It is seen that TAS level is close in the C and CRB groups (p>0.05) and is significantly higher than the N and N+CRB groups (p<0.001).TAS value was significantly highest in the CRB group (p<0.001) and TAS values of the C and CRB groups were calculated close to each other (p>0.05). TOS and OSI values were significantly higher in N group compared to other groups (p<0.001) **(Table 4)**.

Table 4: Biochemical analysis results on oxidative stress parameters and antioxidant enzymes in heart tissue by groups

Parameters\Groups	C	CRB	N	N+CRB	*p*
TBARS	2.89±0.19^{a}	3.21±0.2^{b}	3.63±0.24^{c}	3.26±0.21^{b}	<0.001
SOD	15.5±0.49^{ad}	14.44±0.45^{bd}	17.13±0.84^{c}	14.75±0.49^{d}	<0.001
CAT	12.6±0.66^{ac}	11.17±0.54^{a}	18.1±2.86^{b}	16.21±4.72^{bc}	<0.001
GPx	31.36±0.98^{a}	30.25±0.5^{a}	39.29±4.38^{b}	36.07±0.71^{b}	<0.001
TAS	1.53±0.03^{a}	1.58±0.12^{a}	1.22±0.17^{b}	1.3±0.16^{b}	<0.001
TOS	7.35±0.46^{a}	6.97±0.7^{a}	10.69±1.02^{b}	8.82±0.26^{c}	<0.001
OSI	0.48±0.03^{a}	0.44±0.05^{a}	0.88±0.07^{b}	0.69±0.1^{c}	<0.001
GSH	3.81±0.13^{a}	3.28±0.75^{ab}	2.49±0.8^{b}	3.69±0.56^{a}	0.004
GSSG	0.56±0.05^{ac}	0.5±0.06^{a}	0.69±0.05$_{b}$	0.59±0.04^{c}	<0.001
GSH/GSSG	4.79±0.44^{a}	4.57±1.53^{a}	1.68±1.51^{b}	4.24±0.68^{a}	<0.001
Redox potential	-66.38±0.47^{a}	-63.36±6.27^{a}	-51.66±8.32^{b}	-64.67±3.31^{a}	<0.001

Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different (p < 0.001). C, *control*; CRB, *carob*; N, *nicotine*; N+CRB, *nicotine+carob*; TBARS, *thiobarbituric acid reactive substance;* SOD, *superoxide dismutase;* CAT, *catalase;* GPx, *glutathione peroxidase;* TAS, *total antioxidative status;* TOS, *total oxidative status;* OSI, *oxidative stress index;* GSH, *glutathione;* GSSG, *glutathione disulfide*

The outcomes of the biochemical analysis of liver tissue are shown in Table 5. These data indicate that the C group has the lowest TBARS level (p<0.001). SOD and CAT activities were calculated at the highest value in the K group (p<0.001). Although the group with the highest value of GPx activity is the N + CRB group, there is no significant difference when compared with other groups (p>0.001). When the TAS levels are analyzed, it is seen that they are close to the C and CRB groups, and to N and N + CRB groups (p>0.05). Here, the TAS level is significantly higher in CRB and C groups, respectively (p<0.001). In TOS levels, the group with the highest value is the N+CRB group, but it is observed that it has a close value with the N group (p>0.05). The group with the highest OSI level was the N + CRB group (p<0.001) **(Table 5)**.

Table 5: Biochemical analysis results on oxidative stress parameters and antioxidant enzymes in liver tissue by groups

Parameters\Groups	C	CRB	N	N+CRB	*p*
TBARS	3.07±0.13^{a}	3.65±0.21^{b}	4.2±0.2^{c}	4.05±0.29^{c}	<0.001
SOD	20.77±0.15^{a}	18.7±0.8^{b}	14.78±0.72^{c}	16.56±0.74^{d}	<0.001
CAT	25.19±0.29^{a}	22.19±1.12^{b}	11.34±0.99^{c}	15.61±1.05^{d}	<0.001
GPx	21.06±0.65^{ab}	20.51±0.47^{a}	20.1±1.69^{a}	22.29±0.83^{b}	0.003
TAS	1.46±0.01^{a}	1.51±0.11^{a}	0.51±0.07^{b}	0.6±0.06^{b}	<0.001
TOS	8.79±0.67^{a}	8.99±0.77^{a}	14.84±2.46^{b}	12.79±0.87^{b}	<0.001
OSI	0.59±0.05^{a}	0.59±0.05^{a}	2.94±0.7^{b}	2.15±0.25^{c}	<0.001
GSH	10.33±0.53^{a}	9.14±2.08^{ab}	7.02±1.04^{c}	8.3±0.6^{bc}	<0.001
GSSG	2.51±0.13^{a}	2.27±0.18^{a}	3.13±0.33^{b}	2.67±0.45^{a}	<0.001
GSH/GSSG	2.11±0.31^{a}	2.03±0.89^{a}	0.29±0.6^{b}	1.2±0.72^{ab}	<0.001
Redox potential	-72.82±1.6^{a}	-70.37±6.21^{a}	-59.73±5.14^{b}	-66.44±3.54^{ab}	<0.001

Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different (p < 0.001). C, *control*; CRB, *carob*; N, *nicotine*; N+CRB, *nicotine+carob*; TBARS, *thiobarbituric acid reactive substance;* SOD, *superoxide dismutase;* CAT, *catalase;* GPx, *glutathione peroxidase;* TAS, *total antioxidative status;* TOS, *total oxidative status;* OSI, *oxidative stress index;* GSH, *glutathione;* GSSG, *glutathione disulfide*

Pyramidal Cells in the Hippocampus

The average number of pyramidal cells for each group was calculated. It was found that the control group had an average of 620.578 pyramidal cells. It can be noticed that the nicotine group has less total pyramidal cells than the control group. Between the control group and the other groups, there is a statistically significant difference **(Table 6)**.

Table 6: Number of pyramidal cells in hippocampus CA region by groups

Experimental Groups	Total Pyramidal cell count	Section thickness (µm)	*p*
Control	620.578±4.45	20	0.001
Nicotine	573.623±13.24	20	0.001
Carob	612.324±5.17	20	0.001
Nicotine+Carob	586.978±3.28	20	0.001

ANOVA test p<0.001 was considered statistically significant. The data are expressed as the mean ± standard deviation.

DISCUSSION

In our study on rats, we examined the oxidative and antioxidant states and pyramidal cell numbers in the hippocampus of nicotine-induced oxidative stress in tissues with the optical fractionator method. We also evaluated the possible effects of carob plant extract against the harmful effect of nicotine.

It has been reported that nicotine contained in cigarette smoke is held responsible for the occurrence of many diseases. This has led to different studies on cigarettes and thus on nicotine (28, 29). Some researchers focused on the relationship between nicotine and oxidative stress. In addition, there are studies in the literature where antioxidants are used to treat damage caused by oxidative stress (30 - 32).

The levels of TBARS, TOS, and OSI were found to be significantly higher in the nicotine group when the control group and other groups were compared in our study's lung tissue's biochemical analysis data. TAS and GSH levels are significantly lower. In contrast, TAS and GSH levels were found to be significantly greater in the carob group, while in a 2019 study, the nicotine group had higher MDA levels and lower GSH levels in the lung tissue of rats exposed to ethanol, nicotine, and caffeine compounds than the control group (33). Abdel-Rahman et. al , in their study, investigated both the protective and therapeutic effect of carob plant extract in the lung tissue of male rats exposed to hookah smoke. In the study results, they concluded that the carob plant has a very effective phytomedical effect against the damage of the hookah (34).

In brain tissue, as a result of the data we obtained from the study, other groups were compared according to the control group. Accordingly, TAS level of nicotine group was significantly lower than other groups. In addition, it was concluded that there was no significant difference between the TAS levels of carob and nicotine + carob groups and that they were close to control. The number of pyramidal cells in brain tissue was found to be significantly lower in the nicotine group. In the literature, dark neurons were depicted as being scattered and clustered, frequently undersized and darkly colored, homogenized with loss of distinguishable internal cell features. Additionally, they stated that although abnormal neurons only compensate around 2 percent of the population overall, they predominated in particular restricted areas (35). The average number of pyramidal cells in previous studies varied between 650,000 and 700,000, but in our study, it was a little lower (26, 27). Any changes in these measurement results are thought to be caused by the particle distribution in the Z axis of the tissues in the paraffin block. Any changes in these measurement results are thought to be caused by the particle distribution in the Z axis of the tissues in the paraffin block.

Navarro and Bover (10) is observed an increase in TBARS level in brain cells of rats exposed to nicotine. Similarly, in our study, TBARS level increased in nicotine group compared to control group. As a result of our study, it is seen that the level of TAS in the kidney tissue of the nicotine group is significantly lower than the control and carob groups. In another study, antioxidant properties were emphasized on kidney and liver tissues by decreasing the lipid peroxidation of carob plant (36).

The effects of the fenton reagent on the lipid peroxidation of extracts of Silybum marianum and Ceratonia siliqua were examined by Akkaya and Yilmaz (37) in their study. As a result, it was found that plant extracts reduce lipid peroxidation level and significantly protect unsaturated fatty acids against free radicals (37). We evaluated this effect of carob with the biochemical data we obtained from the heart tissue. Our study's findings revealed that the nicotine group's TOS level increased statistically significantly in comparison to the control group. The levels of TOS in the carob and nicotine + carob groups were found to be considerably lower than those in the nicotine group.

In a study conducted by Temiz et al. (38), they evaluated the antioxidant role of carob and its protective properties on liver tissue against ethanol-induced oxidative stress. They obtained the result that the locust plant brought the imbalance caused by ethanol between the MDA and the fluctuating antioxidant system closer to normal, especially in tissues (38). In another study conducted in 2015, they said that carob plants had an adverse effect on oxidative stress formation by preventing free radical accumulation in liver tissue (14). As a result of the study, we obtained data in the same direction. The OSI value in liver tissue was significantly greater in the nicotine group than in the control group. When compared to the nicotine group, this number was considerably lower in the nicotine + carob group. In another study, it was found that carob plant has an antioxidant effect on stomach tissue (39). Qasem et al. (40), also emphasized the antioxidant properties of this plant.

Rats were exposed to oxidative stress induced on by nicotine, and the antioxidant properties of carob extract were examined. The results show that the carob plant has antioxidant properties against nicotine. Depending on the level of nicotine consumption, the hippocampus exhibited a different number of pyramidal cells.

According to these findings, carob can be considered as an alternate product for smokers and people with diseases causing oxidative stress.

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