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Neuroprotective Activity of Strawberry Tree (*Arbutus unedo* L.) Against Formaldehyde-Induced Oxidative Stress in The Rat Hippocampus

Actividad Neuroprotectora del Madroño (*Arbutus Unedo* L.) contra el Estrés Oxidativo Inducido por Formaldehído en el Hipocampo de Rata

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SUMMARY: Formaldehyde induces oxidative damage in hippocampal tissue and blood serum by suppressing antioxidant defenses and increasing lipid peroxidation. This results in damage to DNA, proteins, and lipids within cells, disrupting neuronal functions, and may even lead to cell death (apoptosis). Apoptosis occurs as a cellular response triggered by formaldehyde through various mechanisms, including mitochondrial dysfunction, oxidative stress, and DNA damage. *Arbutus unedo* L. (AUL), which is rich in antioxidants, represents a potential countermeasure. The aim of our study is to investigate the immunohistochemical and biochemical effects of AUL fruit extract (AUE) on apoptosis and oxidative stress induced by FA exposure in the rat hippocampus. The rats were divided into four groups: the experimental group (EG) received 20 mg/kg AUE by oral gavage and 10 ppm FA inhalation; the formaldehyde group (FG) received 10 ppm FA inhalation only; the sham group (SG) received 10 mg/kg AUE by oral gavage; and the control group (CG) underwent routine observation. Hippocampal tissue was isolated after blood sampling. A number of physical changes, such as yellowing of feathers and tail spots, were observed in FA-exposed groups. The biochemical analysis showed significant differences in brain-derived neurotrophic factor (BDNF), malondialdehyde (MDA), GSH (Glutathione) and nitric oxide (NO) levels between the groups. The AUE-treated groups had reduced MDA levels, suggesting a potential reduction in oxidative stress, along with lower apoptotic cell rates in caspase-3 assessments. The FG rats had lower BDNF levels than CG. The SG had the fewest apoptotic cells. All these findings indicate AUE's potential to mitigate neuron damage and reduce oxidative stress.

KEY WORDS: Apoptosis; *Arbutus unedo* L; Formaldehyde; Hippocampus; Oxidative stress.

INTRODUCTION

AUL is a shrub species that produces red fruits and belongs to the Ericaceae family. The literature reports that AUL fruits contain numerous phenolic acid derivatives (Ayaz *et al*., 2000; Pallauf *et al*., 2008), as well as vitamins E and C and important minerals (Pabuçcuoglu *et al*., 2003; Özcan & Hacıseferogulları, 2007; Olveria *et al*., 2011; Miguel *et al*., 2014) Hence, it can be inferred that AUL fruits possess strong antioxidant properties.

Arbutus unedo L., commonly known as the strawberry tree, is known for its rich content of phenolic compounds. These components include flavonoids and ellagitannins, as well as vitamins with strong antioxidant properties (Miguel *et al*., 2014). A study conducted in the Cáceres region of Spain showed that fully ripened strawberry tree fruits could be classified into seven phenolic subclasses based on spectral identification after separation by high performance liquid chromatography (HPLC): catechins and proanthocyanidins, hy-droxybenzoic acids, ellagitannins, ellagic acid, hydroxycinnamic acids, flavonols and anthocyanins. These phenolic compounds exhibit significant antioxidant activity and have been associated with several

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health benefits, including neuroprotective effects (Olveria *et al*., 2011; Miguel *et al*., 2014). Furthermore, a study conducted in northeastern Portugal identified flavan-3-ols and their galloyl derivatives as the predominant phenolic compounds in wild strawberry tree fruits, followed by anthocyanins and flavonols. In addition, a study using liquid chro-matography coupled with mass spectrometry demonstrated the presence of different flavonols in Arbutus fruits, contributing to their antioxidant capacity (Olveria *et al*., 2011). The presence of these diverse phenolic components highlights the potential health benefits of *Arbutus unedo* L. fruits, including their neuroprotective effects, which warrant further research.

Formaldehyde (HCHO) induces neurotoxic effects by generating excessive intra-cellular reactive oxygen species (ROS). Reactive oxygen species (ROS) are free radicals that originate from oxygen, while reactive nitrogen species (RNS) originate from nitrogen. The increase in ROS during metabolism disrupts the balance between antioxidants and oxidants. Endogenous antioxidants attempt to restore this balance by increasing their levels. However, if the amount of free radicals exceeds the capacity of antioxidants to balance them, oxidative stress occurs. Cell and tissue damage occurs when oxidative stress exceeds the tolerable level, which can be tolerated only to a certain extent (Baek & Lee, 2016). ROS are free radicals produced as part of normal cellular functions. However, when produced un-balanced or not scavenged, ROS can lead to cellular damage and apoptosis (Halliwell, 1989; Valko *et al*., 2007). The role of ROS in the pathogenesis of neurodegenerative diseases is increasingly highlighted. Oxidative stress has an important place among the many possible mechanisms leading to neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases. Signs of oxidative damage such as lipid peroxidation, protein oxidation, and DNA damage caused by ROS have been observed in these diseases (Butterfield & Halliwell, 2019; Butterfield, 2023; Orfali *et al*., 2024). On the other hand, recent research indicates that ROS are not only harmful molecules but also im-portant signaling molecules in cellular signaling pathways (Finkel, 2012; Masuda *et al*., 2024).

Apoptosis is the process of eliminating cells that have completed their function or whose DNA has been damaged, without harming the surrounding cells and tissues. This process occurs throughout life. For instance, during embryonic development, the cells between the fingers and toes undergo programmed cell death, resulting in the separation of the digits. Similarly, the regression of the mammary gland after lactation and organ atrophy in old age are physiological examples of apoptosis. Cell deaths occurring in ischemic diseases, such as radiation, chemotherapy, hypoxia, and myocardial infarction (MI), are defined as pathological apoptosis (Kiess & Gallaher, 1998; Elmore, 2007; Pistritto *et al*., 2016). Formaldehyde is a highly reactive chemical compound in the aldehyde group that causes oxidative stress.

The aim of this study is to investigate the positive effects of AUE, which contains antioxidant compounds, on rat hippocampus subjected to oxidative stress and apoptosis induced by FA, which is known to have negative effects on the nervous system.

MATERIAL AND METHOD

Plant material. *A. unedo* L. is a type of fruit collected from the natural flora in Pamukova. No pesticides were applied while the plant was growing. *A. unedo* L. is a type of fruit that does not have selective soil, The soil where it is collected is alkaline, calcareous and infertile. *A. unedo* L. were collected from Sakarya, Turkey from the Pamukova region in Sakarya, Turkey (latitude: 40° 32´ 45.2328" N; longitude: 30° 11´ 58.9164" E) in November 2021. Since it takes about 12 months for the fruit to mature, ripe fruit is always on the tree. The fruits were collected in November.

Extract preparation. AUL fruits collected from the western Black Sea coast in season were dried and preserved with the freeze-drying method.

The product yield in extraction was determined as 70 %. The volume/size of the freeze-dried fruit sample was not determined before and after freezing.

Sample: methanol was treated at a ratio of 1:10. Mixture, Whatman No. It was filtered under vacuum on a Buchner funnel using 1 paper (Whatman Inc., Clifton, N.J.). The filtrates were evaporated under vacuum at 400 °C on a rotary (HEI-VAP Value G1, Schwabach, Germany). The resulting dry extract was prepared with distilled water at the specified concentration and stored at +40 °C in dark colored bottles. The equipment consisted rapid scanning UV visible photodiode array detector.

HPLC analysis. Reverse phase high performance liquid chromatography method was used for the simultaneous determination of 4 phenolic compounds in *Arbutus unedo* L. extract. The compounds determined were gallic acid, catechin and gallic acid.

The HPLC analysis of phenolic compounds was performed using a 5 μm C18 column (250 mm × 4.6 mm,) thermostated at 30 °C. The equipment consisted rapid scanning UVvisible photodiode array detector. The column

was a Zorbax Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA, USA); (5µm, 4.6mm I.D. x 250 mm) Mobile phase consisted of (A) acetonitrile and (B) formic acid at a flow rate of 1 mL/min.

Total phenolic content. The Folin-Ciocalteu spectrophotometric method was used. According to this method, the sample, Folin reagent and pure water were added to the tubes and allowed to stand for 3 minutes. Then 20 % Na_2CO_3) was added and, after 1 hour in the dark, the absorbance was measured against the blank prepared in the same way at a wavelength of 765 nm in a spectrophotometer (Shimadzu UV-1240). The phenolic content of the samples was calculated from the standard curve prepared with gallic acid. For this purpose, 10 different gallic acid solutions were prepared in the concentration range of 25-1000 mg/L and the absorbance values of these solutions were determined at a wavelength of 765 nm. The total amount of phenolic substances in the samples was calculated as mg GAE/g extract. This analysis was performed in 3 replicates and the results were averaged. Result: TPC (mg GAE/g extract): 49.89 ±1.35.

Chemicals and reagents. All chemicals were of analytical grade. Reduced glutathione (GSH), thiobarbituric acid(TBA), Ethylenediamine tetra acetic acid (EDTA), Disodium phosphate (NA_2HPO_4) were from Sigma-Aldrich (Stein,Germany) and BDNF enzyme-linked immunosorbent assay (ELISA) kit were obtained from Sunred Biological Technology (Shanghai,China). Other reagent, such as formaldehyde, were obtained from (Darmstadt, Germany).

Animals. The study used adult female Wistar rats (Experimental Medical Research and Ap-plication Center, Kocaeli University, Kocaeli, Turkey) weighing between 200 and 350 grams. Only female rats were chosen in our experiment. The primary reason for this choice is our intention to focus on the female reproductive system in subsequent stages. This approach aims to minimize harm to a reduced number of animals within the framework of the 3R principle. The rats were housed in an animal colony at a density of approximately 8 to 9 per cage for 2 weeks prior to the experiments. Before starting the experiment, the rats' body weights were measured and recorded (239.13± 13.26 g). The experiments were conducted in compliance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26220). The Kocaeli University Animal Research Ethics Committee granted ethical approval (Project number: HADYEK 2021/17, Kocaeli, Turkey).

Experimental design. The study randomly selected animals and divided them into four groups, each containing nine rats. The tails of the rats in each group were painted different colors. Group I was designated as the control group (CG), Group II as the experimental group (EG), Group III as the formaldehyde group (FAG), and Group IV as the sham group (SG). The experimental period lasted for five weeks, accounting for inhalation and gavage losses.

Nine rats were assigned to each of the following groups: CG, EG, SG, and FAG. The rats in the CG group were exposed to normal air, while the rats in the EG group were given 20 mg of lyophilized AUE by oral gavage along with 10 ppm FA for 4 hours daily, five days a week. The rats in the SG group were given 10 mg of AU extract by oral gavage on a daily basis for 30 days. The planned experiment to demonstrate the difference between 10 mg (used in the sham group) and 20 mg (used in the experimental group) aims to evaluate in more detail the potential effects of phenolic acids on neuroprotective activity. The results of this experiment may help us better understand the effects of different doses on neuroprotective activity whilst contributing to the literature. The rats in the FAG group were exposed to subacute FA (10 ppm FA) for 4 hours a day, five days a week throughout the experiment.

Experimental chamber. A glass experimental chamber was prepared, with dimensions of 100 cm (length) x 70 cm (width) x 35 cm (height), following Matsuoka's methodology (Matsuoka *et al*., 2010). The chamber was divided into two compartments: the experimental chamber and FA evaporation, as shown in Figure 1.

Formaldehyde is passed through a small hole of 6 cm diameter between the two compartments. The reason for preferring the glass chamber is to be able to monitor the adverse situations that can develop in rats due to exposure to FA, which is highly toxic.

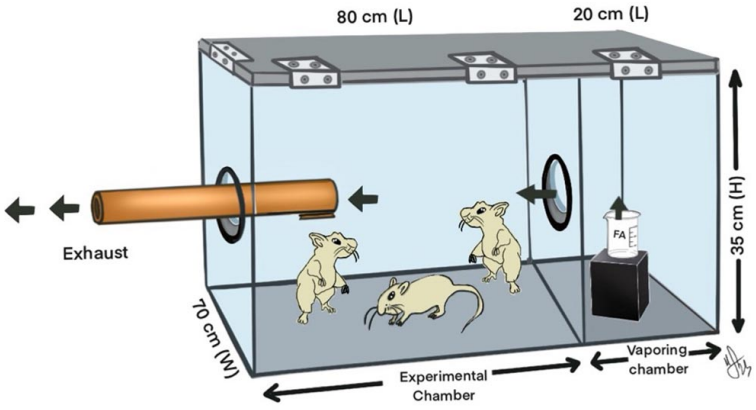

Fig. 1. Illustration showing the experimental chamber system used for FA exposure (drawn by Mehtap Erdogan).

A Honeywell ToxiRAE PRO (HCHO) dosimeter was used to monitor levels of the volatile gas FA throughout the experiment, which lasted an average of 4 hours per day. When the level of FA in the environment fell below 10 ppm, the container of FA was replaced with a new one. To obtain reliable values, the dosimeter was calibrated each day before the start of the experiment.

The experiment was terminated at the end of the fifth week. At the conclusion of the experiment, all the rats were sacrificed after blood sampling under deep anesthesia. Specifically, all the rats were deeply anesthetized with 6 mg/kg Xylazine Hydrochloride (Rompun, Bayer, Istanbul, Turkey) and 30 mg/kg Ketamine Hydrochloride (Ketalar, Parke-Davis, Istanbul, Turkey). Brain tissues were extracted from the sacrificed rats using the isolation technique in accordance with the procedure.

Blood Samples. GSH, NO determination, and BDNF and MDA analysis were performed on the blood samples obtained.

Non-protein sulfhydryl groups are all present in the form of reduced glutathione. DTNB (5,5'-dithiobis (2-nitrobenzoic acid)), a disulfide chromogen, forms a dark yellow compound that can easily be reduced by compounds containing sulfhydryl groups. The absorbance of this reduced chromogen at 412 nm is directly proportional to the concentration of GSH (Ellman, 1959). The GSH analysis method used in the paper is based on this principle.

Plasma BDNF levels were measured using a rat BDNF enzyme-linked immunosorbent assay (ELISA) kit (Sunred Biological Technology, Cat. No. 201-11-0477, Shanghai, China).

To determine MDA levels as an indicator of lipid peroxidation, we utilized the re-action of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a pink-colored complex, measured spectrophotometrically at 535 nm absorbance. This method allowed for accurate quantification of MDA levels in the sample.

For the determination of NO, the ABTS or SULF=NEDD (sulfanilamide) = (N-(1-naphthyl)-ethylenediamine dihydrochloride) method was employed. This method induces a color change in NO solutions and is useful for determining NO concentration. The Ferrosion method is preferred for concentrations above 25 mM, while ABTS is pre-ferred for concentrations below 2 mM (Nims *et al*., 1996). Following the experiment, the sera were stored at40°C and preserved, then analyzed using the Elabscience (Wuhan, China) Rat Galanin ELISA Kit (E-EL-R0396).

Caspase 3 immunohistochemistry. Brain tissues obtained from the animals were fixed in a 10 % buffered formalin so-lution for 3-7 days. After fixation, the brain tissues were dehydrated in increasing concentrations of ethanol and embedded in paraffin. Subsequently, sections with a thickness of 4 mm were cut from the paraffin blocks. Immunohistochemical labeling with a caspase-3 antibody was conducted to detect apoptosis in the hippocampal sections obtained. H-score analysis was performed using the cell numbers obtained from caspase-3 labeling of the groups in the experiment.

To detect apoptosis, the caspase-3 primary antibody (Cell Signaling, #9662) was applied, and immunohistochemical staining was performed. Paraffin sections were de-paraffinized and rehydrated. Antigen retrieval was achieved by boiling the sections in citrate buffer, followed by blocking to prevent non-specific antibody binding. The caspase-3 primary antibody was then incubated overnight at +4 °C. Visualization was performed using DAB chromogen, followed by counterstaining with Mayer's hematox-ylin.

Cells from the hippocampi of four different groups used in the experiment were examined using a Leica DM 1000 LED light microscope.

Statistical analysis. The data were analyzed utilizing the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA).

One-way analysis of variance (ANOVA) was used to compare response groups. Statistical analysis involved the use of the Kruskal-Wallis test to examine significant differences in mean histopathological lesion scores.

RESULTS

HPLC Analyses. The analysis was performed in triplicate, and the averages of gallic acid, epicatechin, catechin and resveratrol were obtained from the values of the three replicates, as shown in Table I.

Histopathological findings of hippocampus tissue. In the hippocampi of rats subjected to oxidative stress and apoptosis induced by FA, neuronal cells of brain tissue were morphologically examined to demonstrate the effects of *Arbutus unedo* L. extract (AUE). Differences were observed in anti-caspase-3 positive staining between the rate of apoptosis in the experimental and sham groups and the rate of apoptotic cells between the FA and control groups (Fig. 2).

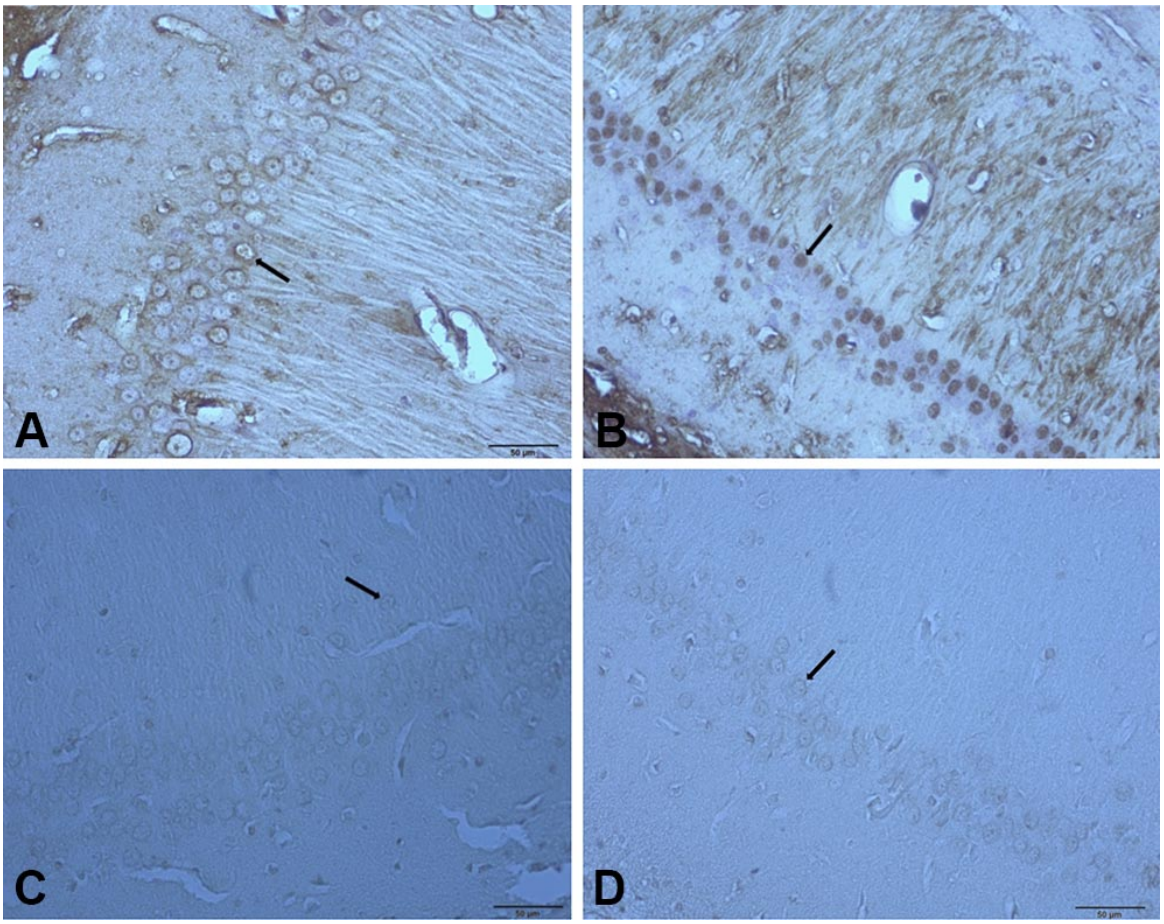

Fig. 2. High-magnification light microscopy images showing representative mi-croscopic H&E staining in the rat hippocampal CA3 regions (scale bar = 20 mm, 400× magnification), arrows indicate hippocampal pyramidal cells. Abbreviations: HE: he-matoxylin-eosin. [a. Experimental group; b. Formaldehyde group; c. Sham group; d. Control group].

Table I. Analytical percentages of antioxidant compounds in *Arbutus unedo* L. extract.

	Gallic acid (mg/kg)	Catechin (mg/kg)	Epicatechin (mg/kg)	t-Resveratrol (mg/kg)
AUL-1	2.276.57	325.11	366.94	0.092
AUL-2	2.124.37	313.10	385.19	---
AUL-3	1.986.86	209.56	345.77	0.085
Mean	2.129.27	282.59	365.97	0.09

In the immunohistochemical images obtained, apoptotic cells surrounded by shrunken membranes are visible in the hippocampal sections of FG and EG rats (Figs. 2a and 2b). The changes in the nuclear structure of these groups in the microscopic images confirm apoptosis and oxidative stress. When the microscopic images of FG and EG rats were compared, it was observed that the cell shape with the specified characteristics was present in FG. According to the H-score values, the number of apoptotic cells is higher in FG than in DG, indicating that AUE has a neuroprotective effect (Fig. 3).

Biochemical analysis results. Serum BDNF, MDA, GSH and NO values were analyzed in the biochemical analysis of rat sera.

In the comparison of MDA, BDNF, NO, GSH and H-score results among the groups, a significant difference was found between the groups in MDA, NO and H-score ($p<0.05$, $p<0.05$ and $p<0.001$) (Table II).

In the pairwise group comparisons of MDA, NO and H-score values, no significant dif-ference was found between the groups for BDNF and GSH ($p>0.05$, $p>0.05$) (Table III).

As a result of pairwise group comparison tests, the MDA values of the sham group were found to be significantly lower than both the experimental and FA groups ($p<0.05$, $p<0.05$). When the average MDA values of the groups are examined, the lowest MDA value is found in the sham group, while the highest MDA value is found in the FA group. The

Table II. Comparison of MDA, BDNF, NO, GSH and H-Score results by groups.

	CG (n=9) mean ± SS	EG (n=9) mean ± SS	FA (n=9) mean ± SS	Sham (n=9) mean. ± SS	p-Value
MDA(_mol/L)	79.32 ± 27.48	84.24 ±15.14	92.64 ± 34.88	71.55 ±15.66	p<0.05
BDNF(pg/mL)	4.43 ± 1.44	4.17 ± 1.37	3.51 ± 0.49	3.38 ± 1.27	p>0.05
NO(_mol/L)	16.04 ± 5.42	12.62 ± 0.65	12.39 ± 0.95	12.83 ± 0.61	p<0.05
GSH(_mol/g)	0.16 ± 0.01	0.15 ± 0.02	0.16 ± 0.03	0.16 ± 0.01	p>0.05
Hscore	37.11 ± 10.01	203.3 ±24.08	334.22 ± 13.132	29.78 ± 6.52	p<0.001

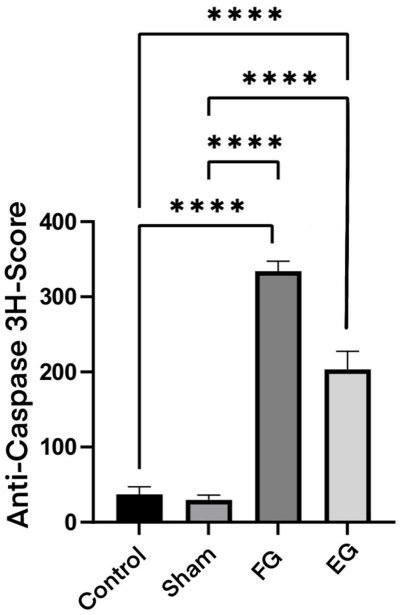

Fig. 3. Anti-Caspase-3 H-Score Levels of the Groups (**** = p<0.0001). [EG:Experimental group; FAG: Formaldehyde group]

Table III. MDA, NO, H-score post-hoc pair group comparison tests.

	MDA	**NO**	**H-score**
Sham-Control	p>0.05	p>0.05	p>0.05
Sham-Experiment	**p<0.05**	p>0.05	**p<0.01**
Sham-FA	**p<0.05**	p>0.05	**p<0.001**
Control-Experiment	p>0.05	**p<0.05**	**p<0.01**
Control-FA	p>0.05	**p<0.01**	**p<0.001**
Experiment-FA	p>0.05	p>0.05	p>0.05

oxidative stress-inducing effect of FA is clear. It was observed that the average MDA values tended to decrease in the sham and experimental groups to which AUE was applied. It was found that the AUE applied to the experimental group tended to reduce the oxidative stress caused by FA, but this reduction was not significant. The MDA level of AUE- applied healthy rats was lower than the control group average, resulting in a significant difference in MDA levels between the experimental and FA groups.

When NO levels are examined, the group with the lowest average is the FA group, whereas the control group exhibits the highest average. The NO levels of the experimental and FA groups were found to be significantly lower than those of the control group (p<0.05, p<0.01). However, as there is no significant difference in NO between the experimental and FA groups, it can be contended that AUE does not contribute to the formaldehyde-induced decrease in NO levels.

The group means for GSH were very close to each other, indicating no significant difference between the groups (p>0.05). It can be argued that GSH levels remained unaffected in rats exposed to the FA environment, and the administration of AUE did not exert a regulatory effect on GSH levels.

Correlation analysis revealed a strong positive correlation between MDA and H-score (p<0.001) (Table IV). The strong positive correlation between the oxidative stress indicator MDA and H-score, representing cell death in brain tissue, suggests that oxidative stress triggers apoptosis in the brain. However, no significant difference was observed between BDNF, NO, and GSH and H-score (p>0.05, p>0.05 and p>0.05, respectively).

Table IV. Correlation of MDA, BDNF, NO, GSH and H-score.

	H-Score
MDA	**KK: 0.558 p <0.001**
BDNF	KK: -0.085 p>0.05
NO	KK: -0.264 p>0.05
GSH	KK: -0.079 p>0.05

DISCUSSION

Inhalation can result in both acute and chronic effects, particularly affecting the respiratory tract, eyes, central nervous system, and skin (Attia *et al.*, 2016). FA can be found

endogenously in living organisms and can also be encountered through environmental exposure (Heck *et al*., 1985). Studies involving FA exposure have demonstrated yellowing of rats' fur due to inhalation (Dubreuil *et al*., 1976), along with behaviors such as avoiding side-to-side movements and huddling together during exposure (Xi *et al*., 2023). Consistent with previous literature, our study observed comparable behaviors, including avoidance, yellowing of feathers, increased eye blinking, and initial reluctance to move. Slowing of movements in the following minutes was also among the physical observations noted.

FA causes toxicological and carcinogenic effects by triggering the formation of reactive oxygen species (Tang *et al*., 2011). A study has shown that FA, which affects many cellular pathways, slows motor activity by causing neurodegenerative changes in the central nervous system (Boja *et al*., 1985). In our study, rats in the groups exposed to FA exhibited normal motor movements before exposure, but slowed movements were observed afterward, confirming this explanation.

BDNF plays an important role in the plasticity, regeneration, and memory functions of brain cells. On average, BDNF levels were lowest in the sham group and highest in the control group. However, no significant difference was observed between the groups. ($p>0.05$) The lower average level of BDNF in the FA group compared to the control group may be associated with impaired memory functions due to FA exposure. It could be suggested that AUE partially mitigates the negative effects of FA on BDNF. However, further studies with larger sample sizes are required to validate this.

In a study where MDA, an indicator of lipid peroxidation and oxidative stress, was used as a parameter, it was observed that the MDA level significantly increased in the FA-exposed group (Zararsiz *et al*., 2006). Similarly, in our study, the highest MDA level was found in the FA group, suggesting that oxidative damage is most pronounced in this group it was observed that the MDA levels tended to decrease in the experimental and sham groups where AUE, a powerful antioxidant, was applied. This suggests that AUE effectively mitigates oxidative damage. The fact that the sham group had the lowest MDA value among all groups indicates the absence of factors currently affecting the balance between ROS and antioxidants. Additionally, it implies that AUE has positive effects on changes occurring in the natural apoptosis process.

One study reported that oxidative stress in brain tissue increased in the group of rats exposed to FA, accompanied by DNA damage (Ciftci *et al*., 2015). Our study similarly found the highest oxidative damage in the FA group. When the H-scores, which express the number of apoptotic cells in the brain, were analyzed, it was found that both the experimental and FA groups had significantly higher scores than the control and sham groups. The highest average number of dead cells was observed in the FA group, and the presence of significantly fewer dead cells in both the sham and control groups compared to the experimental and FA groups suggests formaldehyde-induced damage to the hippocampus. Although the experimental group had a lower average number of dead cells than the FA group, this difference was not found to be significant. While the average number of dead cells in the sham group was lower than the control group, this difference was also not found to be significant. However, it can be suggested that AUE tends to attenuate cell death in the brain.

A study conducted in 2013 reported degenerations related to learning and memory and an increase in the number of apoptotic cells in the hippocampal region in rats exposed to FA (Tang *et al*., 2013). In our study, a correlation was observed between the apoptotic cell index and neuronal damage in the immunohistochemical evaluations of FG and DG due to FA exposure. Apoptotic cell indices in the CA3 region of the hippocampus were found to be significantly higher in FG and DG rats compared to SG and CG.

During apoptosis, the cytoplasm begins to shrink and contract. With the degradation of some structural proteins, the nucleus begins to condense, typically with the nuclear chromatin shifting towards the inner side of the nuclear membrane, taking on a shape similar to a horseshoe or the letter C (Trump *et al*., 1997). In the immunohistochemical images obtained in our study, apoptotic cells surrounded by shrunken membranes were visible in the hippocampal sections of FG and DG rats. The apoptotic changes in the nuclear structure of these groups that were observed through both microscopic imaging and H-score analysis corroborate the aforementioned observations. When the microscopic images of FG and DG rats were compared, it was observed that the cell shape with the specified characteristics was present in FG. According to H-score values, the number of apoptotic cells is higher in FG compared to DG, indicating that AUE has a neuroprotective effect.

The results of our study have highlighted the detrimental effects of formaldehyde exposure on the hippocampus. Formaldehyde exposure has been associated with increased lipid peroxidation, decreased antioxidant defenses, and damage to DNA, proteins, and lipids within cells. This process triggers cellular apoptosis, disrupting neuronal function and even leading to cell death. However, AUE, which is rich in antioxidant compounds, emerges as a potential countermeasure.

Analyses of blood samples revealed a significant decrease in MDA levels among the AUE-treated groups, indicating a potential mitigation of oxidative stress. In addition, caspase-3 assays showed low rates of apoptotic cell ratios. Our results suggest the potential of AUE to alleviate neuronal damage and reduce oxidative stress. The observed reduction in MDA levels following administration of AUL extract is consistent with findings from previous studies, confirming the antioxidant properties of flavonoid aglycones (Sun *et al*., 2011; Cakar *et al*., 2021).

In conclusion, our study demonstrates that formaldehyde exposure increases oxidative stress and apoptosis in hippocampal tissue. However, we have shown that the antioxidant properties of AUE can mitigate these adverse effects. This study contributes to the exploration of natural compounds with protective potential against chemicals, such as formaldehyde, which have detrimental effects on the nervous system.

There is a positive correlation between oxidative stress and GSH levels. Literature on animal studies suggest that as oxidative stress rises, so do GSH levels (Smith *et al*., 1993). However, contrary to these findings, our study revealed minimal variance in mean GSH levels among groups, with no significant differences detected. This suggests that GSH levels remain unaffected in rats exposed to the FA environment, and AUE does not regulate GSH levels.

CONCLUSION

Our study found a correlation between FA exposure and apoptosis, consistent with the literature. A significant increase in the rate of apoptotic cells was observed in hippocampal tissues among the groups exposed to FA compared to other groups.

Biochemical and histological data indicated a reduction in cell death among the AUE-applied groups compared to those not receiving the extract.

FA is a highly reactive free radical commonly used in cadaver embalming laboratories. Literature suggests that occupational groups exposed to FA may experience adverse effects on the nervous system, particularly with chronic exposure. We believe that a comprehensive study of high-antioxidant compounds like AUE, which may minimize the degenerative effects of FA on the hippocampus that is crucial for learning and memory, may prevent cellular-level damage.

ERDOGAN, M.; COLAK, T.; CEYLAN, F. S.; KIR, H. M.; KURNAZ, S.; OZSOY, O.D. & SAHIN, Z. Actividad neuroprotectora del madroño (*Arbutus unedo* L.) contra el estrés oxidativo inducido por formaldehído en el hipocampo de rata. *Int. J. Morphol., 42(5)*:1338-1346, 2024.

RESUMEN: El formaldehído induce daño oxidativo en el tejido del hipocampo y del plasma sanguíneo al suprimir las defensas antioxidantes y aumentar la peroxidación lipídica. Esto provoca daños en el ADN, las proteínas y los lípidos dentro de las células, alterando las funciones neuronales e incluso puede provocar la muerte celular (apoptosis). La apoptosis se produce como una respuesta celular desencadenada por el formaldehído a través de diversos mecanismos, incluida la disfunción mitocondrial, el estrés oxidativo y el daño del ADN. *Arbutus unedo* L. (AUL), rico en antioxidantes, representa una posible contramedida. El objetivo de nuestro estudio fue investigar los efectos inmunohistoquímicos y bioquímicos del extracto de fruta AUL (AUE) sobre la apoptosis y el estrés oxidativo inducido por la exposición a FA en el hipocampo de rata. Las ratas se dividieron en cuatro grupos: el grupo experimental (GE) recibió 20 mg/kg de AUE por sonda oral y 10 ppm de FA por inhalación; el grupo de formaldehído (GF) recibió sólo una inhalación de 10 ppm de FA; el grupo simulado (GS) recibió 10 mg/kg de AUE mediante sonda oral; y el grupo control (GC) se sometió a observación de rutina. Se aisló tejido del hipocampo después de una muestra de sangre. En los grupos expuestos a FA se observaron una serie de cambios físicos, como coloración amarillenta del pelaje y manchas en la cola. El análisis bioquímico mostró diferencias significativas en los niveles de factor neurotrófico derivado del cerebro (BDNF), malondialdehído (MDA), GSH (glutatión) y óxido nítrico (NO) entre los grupos. Los grupos tratados con AUE tenían niveles reducidos de MDA, lo que sugiere una reducción potencial del estrés oxidativo, junto con tasas de células apoptóticas más bajas en las evaluaciones de caspasa-3. Las ratas GF tenían niveles de BDNF más bajos que GC. El GS tenía la menor cantidad de células apoptóticas. Todos estos hallazgos indican el potencial del AUE para mitigar el daño neuronal y reducir el estrés oxidativo.

PALABRAS CLAVE: Apoptosis; *Arbutus unedo* L; Formaldehído; Hipocampo; Estrés oxidativo.

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