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RESEARCH ARTICLE

Investigation of Dimethoate Toxicity in Rat Brain and Protective Effect of *Laurocerasus officinalis* Roem. Fruit Extract Against Oxidative Stress, DNA Damage, and Apoptosis

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Investigation of Dimethoate Toxicity in Rat Brain and Protective Effect of Laurocerasus officinalis Roem. Fruit Extract Against Oxidative Stress, DNA Damage, and Apoptosis

SUMMARY

Dimethoate is an organophosphate insecticide that is used globally on a wide scale in agriculture. It was associated with numerous negative health effects in many studies. The brain is one of the target organs for dimethoate exposure. The present study aimed to evaluate the sub-chronic (60 days) toxicity of dimethoate (7 mg/kg body weight) by investigating its oxidative stress, DNA damage, apoptosis-inducing effects, and histopathological changes in brain tissue of rats. We also aimed to analyze the protective effects of Laurocerasus officinalis Roem. (cherry laurel) fruit extract. To evaluate oxidative stress, malondialdehyde (MDA) levels, as well as superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GPx), and catalase (CAT) antioxidant enzymes activities, were calculated. Experimental results demonstrated that dimethoate treatment increased MDA and decreased Cu-Zn SOD, GPx, and CAT enzyme activities, suggesting its potency as an oxidative stress inducer in rat brain tissues. Furthermore, comet and TUNEL assay results showed that dimethoate stimulated DNA damage and apoptosis. Administration of cherry laurel extracts protected against dimethoate-induced oxidative stress, DNA damage, and apoptosis. The findings of the current study are important in terms of demonstrating the beneficial effects of L. officinalis extract against dimethoate toxicity in the brain, considering the sensitivity of this organ to oxidative stress and extensive usage of dimethoate.

Key Words: *Dimethoate, neurotoxicity, Laurocerasus officinalis Roem., in vivo, DNA damage, oxidative stress*

Dimetoat Toksisitesinin Sıçan Beyninde Araştırılması ve Laurocerasus officinalis Roem. Meyve Ekstresinin Oksidatif Stres, DNA Hasarı ve Apoptoza Karşı Koruyucu Etkisi

ÖZ

Dimetoat dünyada tarımda yaygın olarak kullanılan organofosfatlı bir insektisittir. Bir çok çalışmada dimetoatın negatif sağlık etkileriyle ilişkili olduğu belirtilmiştir. Beyin, dimetoat maruziyeti için başlıca hedef organlarından birisidir. Bu çalışmadaki amacımız dimetoatın (7 mg/kg vücut ağırlığı) sub-kronik (60 günlük) toksisitesini, sıçan beyin dokuları üzerindeki oksidatif stres, DNA hasarı ve apoptoz indükleyici etkilerini ve histopatolojik değişiklikleri araştırarak değerlendirmektir. Ayrıca Laurocerasus officinalis Roem. (taflan) meyve ekstresinin koruyucu etkilerinin incelenmesi hedeflenmektedir. Oksidatif stres değerlendirmesi için malondialdehit düzeyleri (MDA) ile birlikte süperoksit dismutaz (Cu-Zn SOD), glutatyon peroksidaz (GPx) ve katalaz (CAT) antioksidan enzim aktiviteleri ölçülmüştür. Deney sonuçları dimetoat muamelesinin MDA'yı arttırdığını ve Cu-Zn SOD, GPx ve CAT enzim aktivitelerini azalttığını göstererek, sıçan beyin dokularında etkin bir oksidatif stres indükleyici olabileceğini ortaya koymaktadır. Ayrıca comet ve TUNEL deney sonuçları da dimetoatın DNA hasarı ve apoptozu arttırdığını göstermiştir. Taflan ekstresi verilmesi, dimetoat tarafından indüklenen oksidatif stres, DNA hasarı ve apoptoza karşı koruyucu etki sağlamıştır. Bu çalışmada elde edilen bulgular, beynin oksidatif strese duyarlılığı ve dimetoatın yaygın kullanımı göz önüne alındığında, L. officinalis ekstresinin dimetoat toksisitesine karşı beyinde faydalı etkilerini göstermesi açısından önem taşımaktadır.

Anahtar Kelimeler: *Dimetoat, nörotoksisite, Laurocerasus officinalis Roem., in vivo, DNA hasarı, oksidatif stres*

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INTRODUCTION

Dimethoate/DMT is an organophosphate insecticide that is widely used in agriculture to protect plants against the harm of insects and mites (Özkara et al., 2016). It acts by inhibiting acetylcholinesterase, an essential enzyme for the normal function of the nervous system (Grue et al., 1997; Eken, 2021). Because of its persistence in soil and plants, widespread usage of dimethoate became a risk for human and animal health (Sharma et al., 2005b; Ahmad et al., 2022), with residues of this pesticide on fruits, vegetables, and drinking water can become chronic exposure sources (Jiang et al., 2022). Previous studies reported that dimethoate had toxicity on various organs and systems, including the liver, lung, kidney, reproductive and immune systems (Bakır et al., 2020; Eken, 2021). Although dimethoate was banned in Europe, it is still being used in several countries (USA, Brazil, etc.) (Ahmad et al., 2022). Recently, the usage of dimethoate was restricted in Turkey (T.C. TOB, 2022).

The brain is considered one of the primary target organs for dimethoate and other organophosphates (Yahia & Ali, 2018). Dimethoate is bioactivated to more neurotoxic omethoate by the cytochrome P450 (CYP 450) enzyme system (Buratti & Testai, 2007; Ahmad et al., 2022). In animal studies, it was shown to cause oxidative stress, inflammation, and mitochondrial function damage in mice and rat brains (Sharma et al., 2005a, 2005b; Astiz et al., 2009b, 2013). The brain is especially susceptible to oxidative damage due to having high levels of polyunsaturated fatty acids (PUFAs) and insufficient free radical scavenging capability (Astiz et al., 2009a).

Interest in studying the preventive effects of natural antioxidants, which are found in several plants, against oxidative stress has increased over recent years (Moure et al., 2001; Ahmadipour et al., 2021). *Laurocerasus officinalis* Roem. (cherry laurel; "taflan, karayemis or laz kirazi" in Turkish) is such a plant native to the Black Sea coast of Turkey (Akkol et al., 2012). Its fruits contain several antioxidant substances, such as phenolics and ascorbic acid (Kolayli et al., 2003; Alasalvar et al., 2005; Karahalil & Şahin, 2011; Orhan & Akkol, 2011). Furthermore, it was previously reported that *L. officinalis* scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide radicals (Kolayli et al., 2003; Orhan & Akkol, 2011). It is known to have diuretic and anti-diabetic properties, as well as several beneficial effects against several illnesses, including gastrointestinal system problems, bronchitis, eczemas, and hemorrhoids (Kolayli et al., 2003). To the best of our knowledge, the protective effects of this extract against *in vivo* dimethoate toxicity in the brain were not previously investigated.

Considering the above-mentioned information, we aimed to evaluate whether sub-chronic dimethoate exposure affects oxidative stress, apoptosis, DNA damage, and histopathological alterations in brain tissue. Moreover, we investigated for the first time the potential protection of *L. officinalis* (LO) extract in the brain against these toxic effects induced by dimethoate *in vivo*.

MATERIALS AND METHODS

Chemicals

A commercial formulation of dimethoate (Korumagor 40 EC, 40%) was bought from Koruma Tarım (Kocaeli, Turkey). All the other chemicals were obtained from Sigma-Aldrich/Merck KGaA (Darmstadt, Germany) unless indicated otherwise.

Collection of plants and fruit extract preparation

Cherry laurel fruits were collected from the Akçaabat district of Trabzon province. The plant was identified by Dr. Ayşe Baldemir Kılıç. The herbarium specimen of the plant was vouchered in Ankara University Faculty of Pharmacy Herbarium (AEF 26257). Fruit extracts were prepared similarly to the protocol described by Bakır et al. (Bakır et al., 2020). Briefly, after drying, the fruit pulp was cut into small pieces and macerated with methanol at room temperature. After

constant stirring and filtration two times, a vacuum with a rotary evaporator was used for drying filtrates. Following lyophilization, lyophilisates were kept at 4°C until administration to animals in 0.9% NaCl.

Animals and experimental design

Male Wistar albino rats weighing 200-250 g were used in the current study. Animals were housed at 22-24°C with 55-60% relative humidity on a 12 h light/dark cycle in Erciyes University Animal Care Unit. Rats had free access to standard laboratory chow and tap water. This protocol was approved by Erciyes University Animal Ethics Committee (Decision Number: 12/82 and date 15.08.2022). All animal procedures complied with directive 2010/63/EU. Sixty rats were divided into six groups, with ten animals in each. All compounds, freshly dissolved in saline (0.9% NaCl), were administered to animals by oral gavage at indicated doses in 1 ml saline for 60 days. Doses were selected as 7, 100, and 4 mg/kg body weight (b.w) for dimethoate, vitamin C and *L. officinalis* extract, respectively, based on doses used in other studies (Pourmorad et al., 2006; Sayim, 2007; Saafi-Ben Salah et al., 2012). To evaluate its protective effects, LO extract was administered to two groups as either pre- or post-dimethoate treatment. The dimethoate dose used in our study, which is in line with doses in other sub-chronic dimethoate neurotoxicity studies in rats (Sharma et al., 2005b; Astiz et al., 2009a,; Arnal et al., 2019), corresponds to approximately 2% of its LD_{50}, considering LD_{50} value for dimethoate is 310 mg/kg (Eken, 2021).

Experimental groups were determined as follows:

Group 1: Control; administered 1 ml/day saline daily for 60 days, per oral (p.o.)

Group 2: DMT; administered 7 mg/kg/day dimethoate in saline for 60 days, p.o.

Group 3: LO; received 4 mg/kg/day LO fruit extract in saline for 60 days, p.o.

Group 4: LO_{pre} + DMT; administered LO fruit extract 30 min before dimethoate treatment for 60 days, p.o.

Group 5: Vit C + DMT; administered 100 mg/kg/day vitamin C in saline 30 min before dimethoate treatment for 60 days, p.o.

Group 6: DMT + LO_{post}; given dimethoate alone for 30 days, and then, dimethoate 30 min before LO fruit extract for the next 30 days, p.o.

All animals were sacrificed 24 h after the last dose under ketamine-xylazine (75 and 8 mg/kg b.w., respectively) anesthesia. Whole brains were removed and rinsed with ice-cold saline. Small sections of the tissues were fixed in formalin for histopathological examination. For the comet assay, sliced parts of brain tissue were put into cold phosphate buffer saline to get cell suspension immediately. The rest of the tissues were stored at -80 °C until analysis of oxidative stress parameters.

Determination of oxidative stress-related parameters

Brain tissues were homogenized in cold 1.15% KCl with a homogenizer (IKA Ultra-Turrax T10, Germany) to obtain 10% (w/v) whole homogenate. Malondialdehyde (MDA) was determined by studying a portion of the whole homogenate. The remaining part of the homogenate was centrifuged at 20,000 × *g* for 25 minutes at 4 °C, and enzyme activities were determined by using obtained supernatant. Lipid peroxidation was determined spectrophotometrically based on the method of Ohkawa et al. (1979). Thiobarbituric acid-reactive substances level was indicated as MDA nmol/mg protein. To determine superoxide dismutase (Cu-Zn SOD) activities, the method of Arthur and Boyne (1985) was used with slight changes. Glutathione peroxidase (GPx) activity was measured by the method of Pleban et al. (1982), and the method of Aebi (1984) was used for the measurement of catalase (CAT) activity. The determination of protein contents of tissue homogenates and supernatants was made based on the method of Lowry et al. (1951). Enzyme activity results were given as U/mg protein.

Evaluation of DNA damage with the comet assay

The comet assay was conducted in brain samples according to the protocol of Singh et al. (1988) with some modifications. Briefly, the single-cell suspension of brain tissues was embedded in 0.8% low-melting agarose on precoated slides with 0.5% normal-melting agarose. After lysing, unwinding of DNA, and electrophoresis steps, the slides were stained with ethidium bromide. To visualize DNA damage, one hundred cells from two replicate slides were examined under a fluorescent microscope (Olympus, BX51, Japan). To evaluate the images, comet assay software (CASP-1.2.2) was used. Expression of DNA damage was made as DNA percentage in tail (tail intensity).

Evaluation of apoptosis with TUNEL staining

Apoptosis was detected with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay with In situ Cell Death Detection Kit (Roche, UT, USA) following the manufacturer's instructions. Paraffin sections from the tissues were deparaffinized, rehydrated, and washed in phosphate buffer saline. After adding the TUNEL reaction mixture to specimens in a humidified chamber, slides were incubated with converter reagent and then with fast red substrate solution (F4648, Sigma). TUNEL-positive cells were identified and counted in ten randomly chosen visual fields in each slide using Image J software at an original x400 magnification. Examination of slides was made with an Olympus BX51 microscope (Tokyo, Japan).

Histopathologic assessment

Brain tissues were fixed in formalin (10%) and embedded in paraffin. Paraffin portions were sliced into 5 μm thickness and dyed with hematoxylin and eosin. They were observed under a light microscope (Olympus BX-51, Japan) equipped with an Olympus DP-71 (Japan) camera at ×20 magnification for histopathological changes.

Statistical analysis

All results were given as mean ± standard error of the mean (SEM). Analysis of statistical difference was made through one-way ANOVA and Dunnett's or Bonferroni's Multiple Comparison post-hoc test with GraphPad Prism 9 (San Diego, CA). $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Oxidative stress is the underlying mechanism for several diseases, including neurotoxicity (Rahal et al., 2014). It happens when an imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity occurs in a biological system (Sies, 1997). In the current study, MDA level was used as an indicator of lipid peroxidation, whereas SOD, GPx, and CAT enzyme activities were measured as cellular defense mechanisms. These three enzymes are among the first lines of cellular defenses against oxidative stress (Ighodaro & Akinloye, 2018). As shown in Figure 1, dimethoate exposure led to a 73% increase in MDA level relative to control ($p<0.001$). All three enzyme activities (Cu-Zn SOD, GPx, CAT) decreased by 25% ($p<0.01$), 45% ($p<0.001$), and 31% ($p<0.05$), respectively, after dimethoate treatment. Pre-treatment with both antioxidants ameliorated MDA induction caused by dimethoate by 35% ($p<0.001$). Furthermore, LO post-treatment resulted in a significant 43% reduction in MDA levels ($p<0.001$). In terms of enzymes, all antioxidant treatments rescued Cu-Zn SOD, GPx, and CAT activities disturbed by dimethoate. The range of increases in the enzyme activities was 32-34% for Cu-Zn SOD ($p<0.01$), 57-65% for

GPx (p<0.001), and 45-56% for CAT (p<0.05).

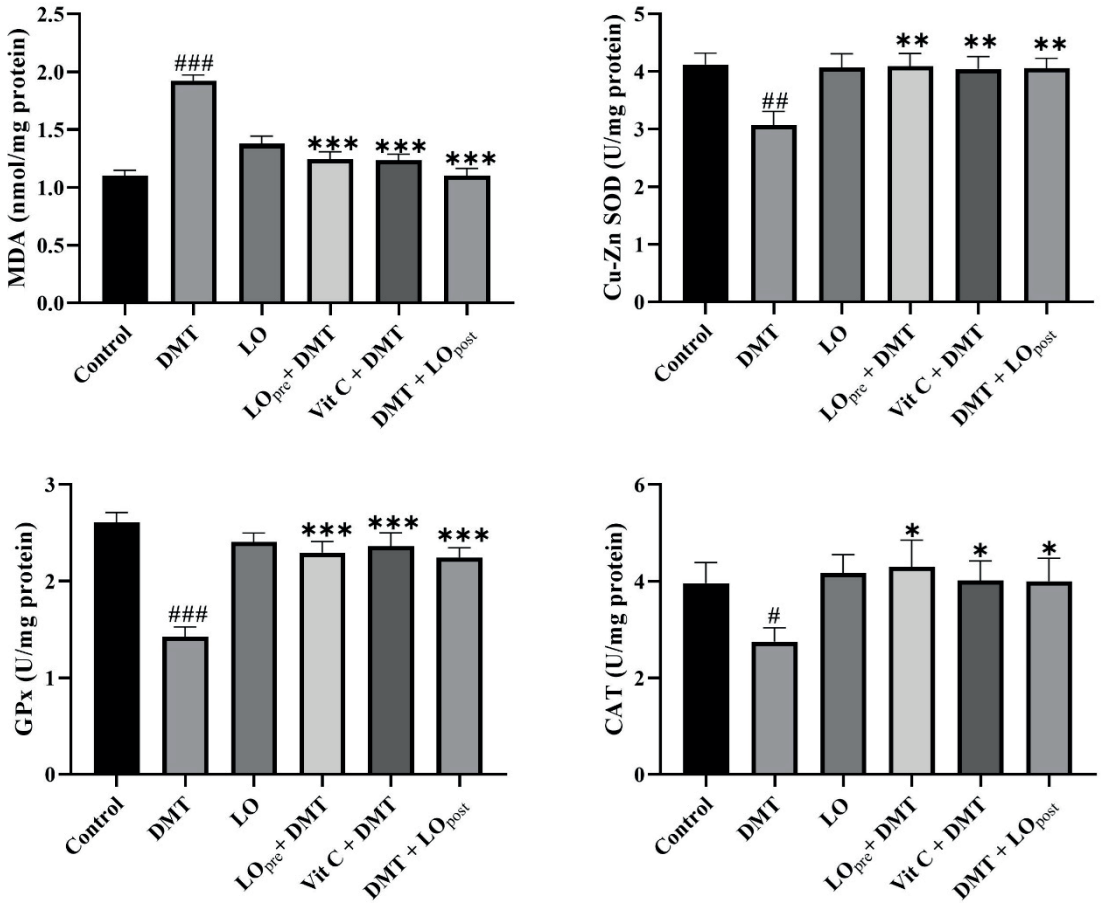

Figure 1. Levels of oxidative stress-related parameters in rat brain tissues given DMT, LO, and Vit C. Data were shown as mean ± SEM. $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 relative to control; *p<0.05, **p<0.01, ***p<0.001

relative to DMT group. DMT: dimethoate, LO: *L. officinalis* fruit extract, Vit C: vitamin C.

As stated before, the nervous system is sensitive to oxidative stress-induced lipid peroxidation because of its containing large amounts of PUFAs and low antioxidant capacity (Astiz et al., 2009a; Endirlik et al., 2022). Dimethoate administration led to increased MDA and depressed SOD, GPx, and CAT, which suggested it had the potency to induce oxidative damage in the brain tissues of rats. Under normal conditions, an equilibrium exists between radical production and antioxidant enzyme activities. When excess ROS are produced in response to chemical exposure, this equilibrium changes, depleting the body's antioxidant defenses (Rahal et al., 2014). Arnal et al. (2019) found that dimethoate exposure elevated MDA levels in rat brains, in agreement with our results. Likewise, in another study with rat brains, dimethoate induced MDA formation, along with reduced SOD activity (Astiz et al., 2009a). In two different studies by Sharma et al. (2005a, 2005b), acute and sub-chronic dimethoate treatment induced lipid peroxidation and histopathological alterations in the brain, whereas SOD, GPx, and CAT activities were found to be augmented, in

contrast to our results. As mentioned before, dimethoate is metabolized by CYP 450 enzymes, and ROS were formed as a by-product of this process (Loida & Sligar, 1993). Moreover, dimethoate was demonstrated to induce CYP 450 isoenzymes, thus leading to further ROS production and subsequent lipid peroxidation (Sharma et al., 2005b).

Oxidative stress and excessive ROS generation might cause oxidative alterations, such as strand breaks, in DNA, (Risom et al., 2005; Kryston et al., 2011). In this study, dimethoate treatment caused a 67% increase in % DNA in tail, relative to the control (p<0.001) (Figure 2). Both antioxidants were shown to protect against dimethoate-induced DNA damage, with LO having more protection than Vit C (38% vs. 24%, relative to the DMT group). Our findings agree with another study where dimethoate exposure caused DNA damage at 48 h in the brain tissue of rats, in addition to MDA induction and GPx decrease (Yahia & Ali, 2018). Previously, Dogan et al. (2011) showed that dimethoate induced DNA damage in *Oncorhynchus mykiss* erythrocytes, which was discussed as being associated with oxidative stress-generating and alkylating properties of this chemical.

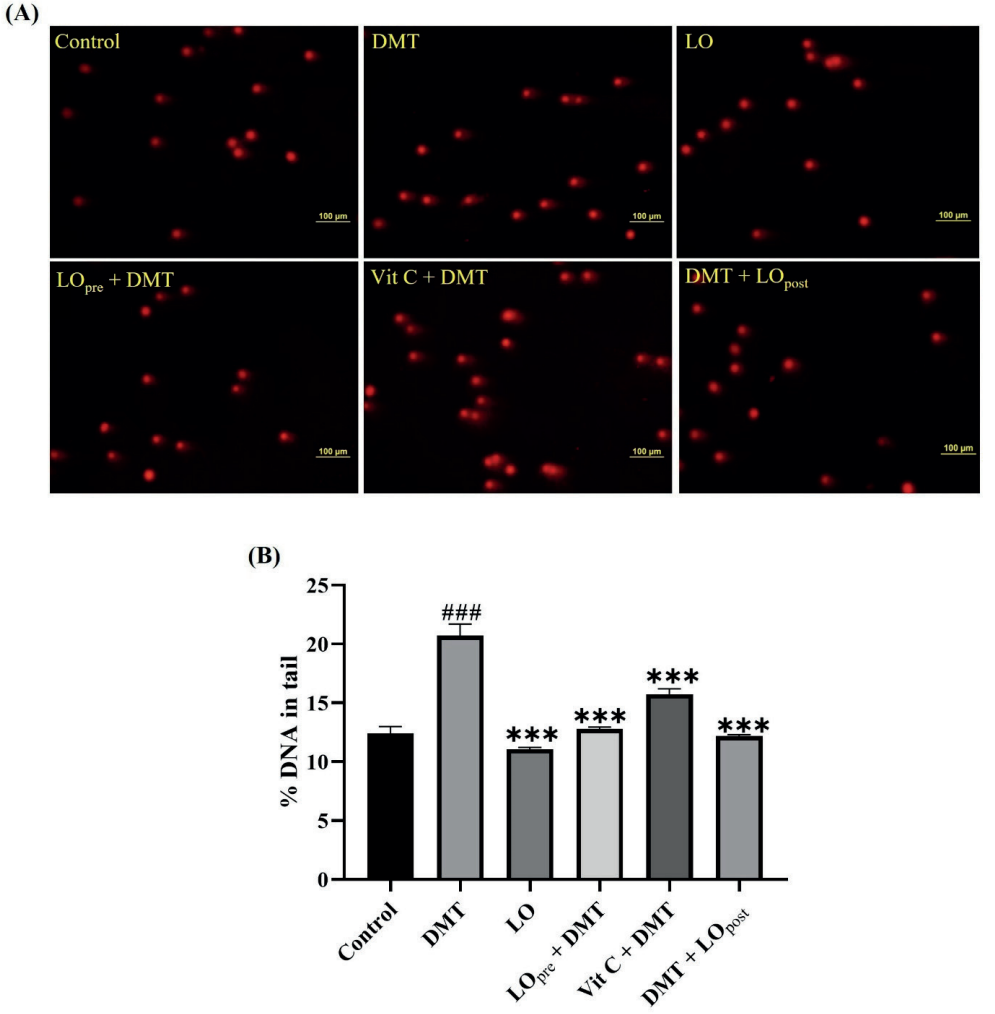

Figure 2. Evaluation of DNA damage in rat brains with comet assay after administration of DMT, LO, and Vit C. (A) Representative images of brain cells stained with ethidium bromide (× 200 magnification). (B) Bar graph data of results obtained from comet assay. Data were shown as mean ± SEM. $^{###}p$<0.001 compared to control; $^{***}p$<0.001 compared to DMT group. DMT: dimethoate, LO: *L. officinalis* fruit extract, Vit C: vitamin C.

Apoptosis is the programmed death of cells, characterized by changes in cellular morphology. The production of ROS and the resulting oxidative stress might play a critical role in regulating apoptosis pathways (Kannan & Jain, 2000). As shown in Figure 3, our results indicated that the TUNEL+ cell percentage increased 3.75-fold after single dimethoate treatment relative to control (p<0.001). Pre-treatments with vitamin C and *L. officinalis* rescued some of the TUNEL+ cell percentage caused by dimethoate by 46% and 53%, respectively (p<0.05), whereas post-treatment of *L. officinalis* was not found to have any significant protective effect. In a study with rat brains, dimethoate exposure for 5 weeks (15 mg/kg b.w.) was also shown to activate intrinsic apoptotic pathways (Arnal et al., 2019). Regarding histopathological results, the cerebrum of brain tissues showed normal histologic architecture with the cell bodies of nerves in all the groups except the DMT group (Figure 4). In this group, nuclear pyknosis was observed in cerebrum neurons. In another study made with rats, sub-chronic dimethoate (30 mg/kg) exposure resulted in mild ischemic changes (Sharma et al., 2005b). The higher dimethoate concentration used in this study might have caused the difference from our research.

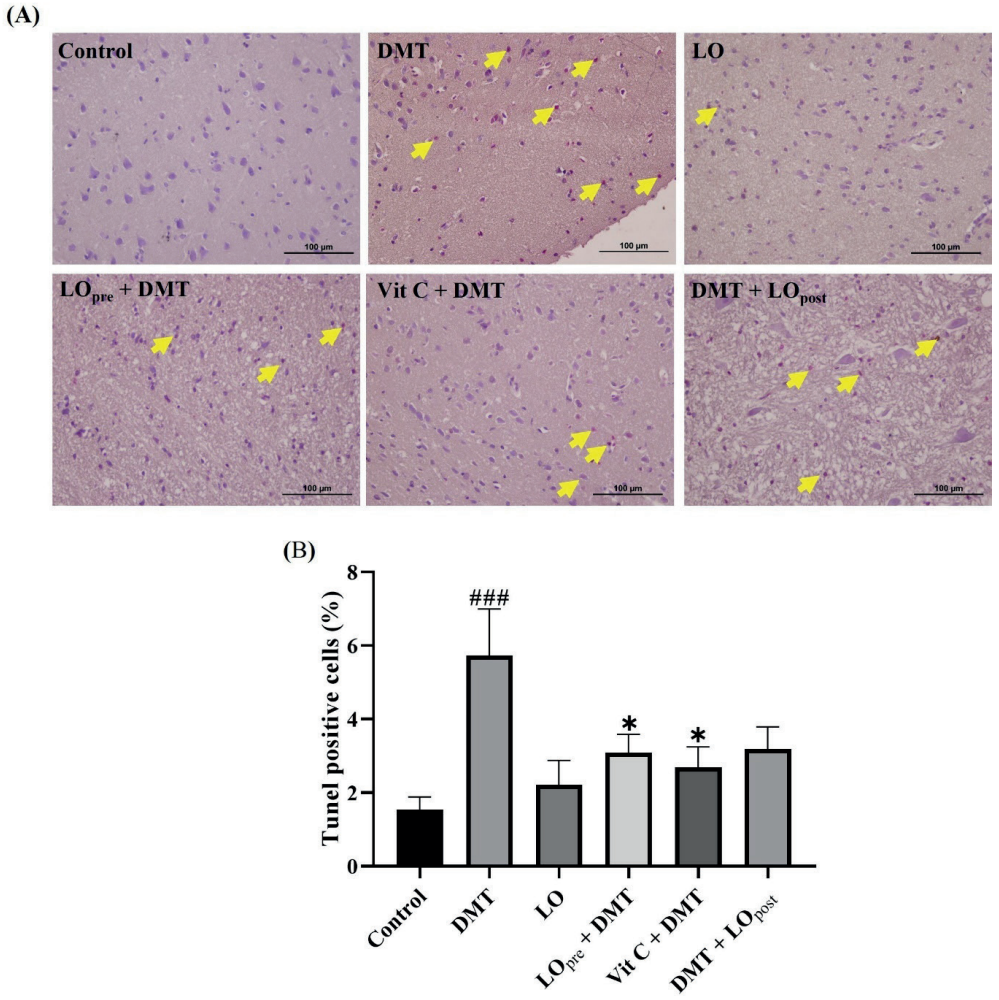

Figure 3. Apoptosis evaluation in brain tissue after administration of DMT, LO, and Vit C (A) Representative images of TUNEL assay (× 400 magnification). Yellow arrows denote TUNEL + cells. (B) Semi-quantitative analysis of TUNEL + cells. Data were shown mean ± SEM. $^{###}p$<0.001 compared to control; $^{*}p$<0.05 compared to DMT group. DMT: dimethoate, LO: *L. officinalis* fruit extract, Vit C: vitamin C.

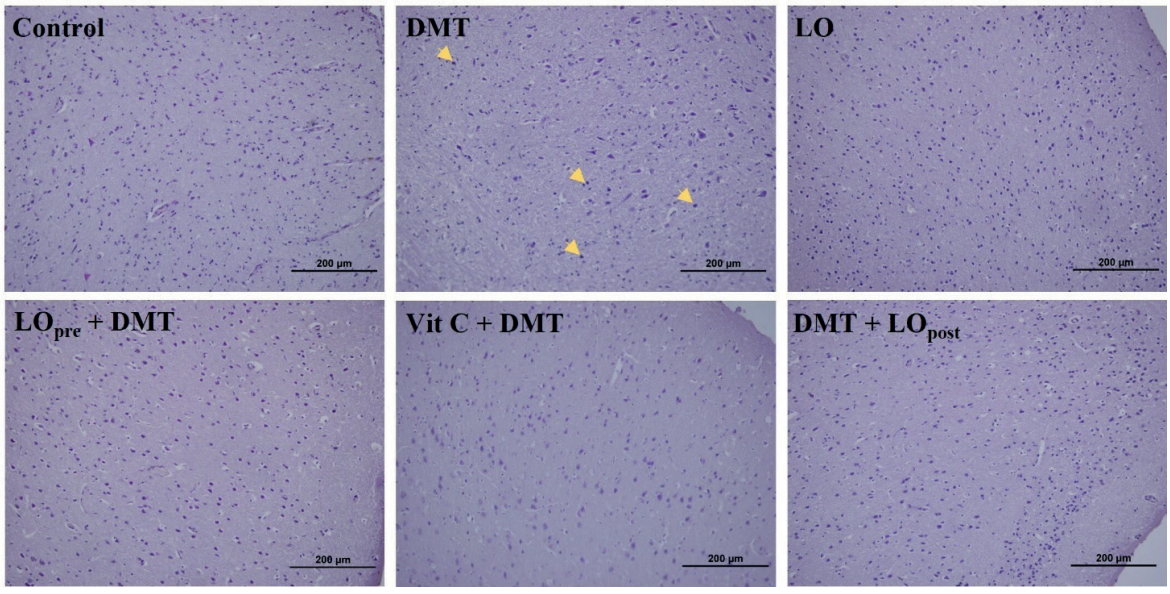

Figure 4. Histopathological examination of brain tissue in rats given DMT, LO, and Vit C. Yellow arrows indicate nuclear pyknosis (× 20 magnification). DMT: dimethoate, LO: *L. officinalis* fruit extract, Vit C: vitamin C.

L. officinalis fruit extract was observed to provide significant protection against lipid peroxidation, DNA damage, and apoptosis, in addition to the restoration of antioxidant enzyme activities close to control levels. These results might be attributed to the antioxidant characteristics of LO extracts. Previously, we demonstrated fruit extract of this plant has DPPH radical scavenging activity and high content of phenolic compounds, which points to its antioxidant property (Eken et al., 2017). Additionally, we used vitamin C as a positive control (Bendich et al., 1986) in our experiments which also demonstrated protection against the harmful effects of dimethoate.

CONCLUSION

In conclusion, we demonstrated lipid peroxidation, DNA damage, apoptosis, and depletion of antioxidant enzymes caused by dimethoate in the brain tissue of rats. Results obtained in the present study are significant, considering the sensitivity of the nervous system to organophosphate insecticide dimethoate exposure and the widespread usage of this chemical in agriculture, especially for occupationally exposed populations. To the best of our knowledge, the antioxidant effects of *L. officinalis* fruit extract against dimethoate-induced neurotoxicity were shown for the first time *in vivo*. However, further studies are required to confirm our findings, with a detailed analysis of the role of components in the beneficial effects associated with this plant extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Concept and Design (A.E.), Supervision (A.E.), Funding Acquisition (A.E.), Materials (B.Ü.E., E.B., A.H.Y., F.C.T., A.B.K.), Data Collection and/or Processing (B.Ü.E., E.B., A.H.Y., F.C.T., A.B.K., A.E.), Analysis and/or Interpretation (B.Ü.E., E.B., A.H.Y., F.C.T., A.B.K., A.E.), Literature Review (B.Ü.E., A.E.) Writing Manuscript (B.Ü.E., A.H.Y., F.C.T., A.B.K., A.E.); Review and Editing (B.Ü.E., E.B., A.H.Y., F.C.T., A.B.K., A.E).

REFERENCES

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105,* 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Ahmad, S., Pinto, A. P., Hai, F. I., Badawy, M. E.T. I., Vazquez, R. R., Naqvi, T. A., Munis, F. H., Mahmood, T., & Chaudhary, H. J. (2022). Dimethoate residues in Pakistan and mitigation strategies through microbial degradation: a review. *Environmental Science and Pollution Research*, *29*, 51367–51383. https://doi.org/10.1007/s11356-022-20933-4

Ahmadipour, A., Sharififar, F., Anani, H., & Karami-Mohajeri, S. (2021). Protective Effects of Ferulic Acid Against Isoniazid-Induced Hepatotoxicity in Rats. *FABAD Journal of Pharmaceutical Sciences*, *46*(2), 119-128.

Akkol, E. K., Kırmızıbekmez, H., Küçükboyacı, N., Gören, A. C., & Yesilada, E. (2012). Isolation of active constituents from cherry laurel (Laurocerasus officinalis Roem.) leaves through bioassay-guided procedures. *Journal of Ethnopharmacology*, *139*(2), 527-532. https://doi.org/10.1016/j.jep.2011.11.043

Alasalvar, C., Al-Farsi, M., & Shahidi, F. (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. *Journal of Food Science*, *70*(1), S47-S52. https://doi.org/10.1111/j.1365-2621.2005.tb09064.x

Arnal, N., Morel, G., Marra, C. A., & Astiz, M. (2019). Pro-apoptotic effects of low doses of dimethoate in rat brain. *Toxicology and Applied Pharmacology*, *363,* 57-63. https://doi.org/10.1016/j.taap.2018.11.013

Arthur, J., & Boyne, R. (1985). Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. *Life Sciences, 36*(16), 1569-1575. https://doi.org/10.1016/0024-3205(85)90381-9

Astiz, M., de Alaniz, M. J., & Marra, C. A. (2009a). Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environmental Toxicology and Pharmacology*, *28*(3), 465-473. https://doi.org/10.1016/j.etap.2009.07.009

Astiz, M., de Alaniz, M. J., & Marra, C. A. (2009b). Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicology and Environmental Safety*, *72*(7), 2025-2032. https://doi.org/10.1016/j.ecoenv.2009.05.001

Astiz, M., Diz-Chaves, Y., & Garcia-Segura, L. M. (2013). Sub-chronic exposure to the insecticide dimethoate induces a proinflammatory status and enhances the neuroinflammatory response to bacterial lypopolysaccharide in the hippocampus and striatum of male mice. *Toxicology and Applied Pharmacology*, *272*(2), 263-271. https://doi.org/10.1016/j.taap.2013.07.008

Bakır, E., Sarıözkan, S., Endirlik, B. Ü., Kılıç, A. B., Yay, A. H., Tan, F. C., Eken, A., & Türk, G. (2020). Cherry laurel fruit extract counters dimethoate-induced reproductive impairment and testicular apoptosis. *Archives of Industrial Hygiene and Toxicology*, *71*(4), 329-338. https://doi.org/10.2478/aiht-2020-71-3412

Bendich, A., Machlin, L., Scandurra, O., Burton, G., & Wayner, D. (1986). The antioxidant role of vitamin C. *Advances in Free Radical Biology & Medicine*, *2*(2), 419-444. https://doi.org/10.1016/S8755-9668(86)80021-7

Buratti, F. M., & Testai, E. (2007). Evidences for CYP3A4 autoactivation in the desulfuration of dimethoate by the human liver. *Toxicology*, *241*(1-2), 33-46. https://doi.org/10.1016/j.tox.2007.08.081

Dogan, D., Can, C., Kocyigit, A., Dikilitas, M., Taskin, A., & Bilinc, H. (2011). Dimethoate-induced oxidative stress and DNA damage in Oncorhynchus mykiss. *Chemosphere, 84*(1), 39-46. https://doi.org/10.1016/j.chemosphere.2011.02.087

Eken, A. (2021). *Dimethoate organophosphate insecticide toxicity and the role of oxidative stress. Toxicology: Oxidative Stress and Dietary Antioxidants.* U.K., Academic Press. https://doi.org/10.1016/B978-0-12-819092-0.00007-8

Eken, A., Endirlik, B., Bakir, E., Baldemir, A., YAY, A., & Canturk, F. (2017). Effect of Laurocerasus officinalis Roem.(cherry laurel) fruit on dimethoate induced hepatotoxicity in rats. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *23*(5), 779-787. https://doi.org/10.9775/kvfd.2017.17748

Endirlik, B. Ü., Eken, A., Canpınar, H., Öztürk, F., & Gürbay, A. (2022). Perfluorooctanoic acid affects mouse brain and liver tissue through oxidative stress. *Archives of Industrial Hygiene and Toxicology*, *73*(2), 148-156. https://doi.org/10.2478/aiht-2022-73-3629

Grue, C. E., Gibert, P. L., & Seeley, M. E. (1997). Neurophysiological and behavioral changes in non-target wildlife exposed to organophosphate and carbamate pesticides: thermoregulation, food consumption, and reproduction. *American Zoologist*, *37*(4), 369-388. https://doi.org/10.1093/icb/37.4.369

Ighodaro, O., & Akinloye, O. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Jiang, Q., Qi, X., Ding, C., Liu, X., Lei, Y., Li, S., & Cao, Z. (2022). Melatonin Rescues Dimethoate Exposure-Induced Meiotic and Developmental Defects of Porcine Oocytes. *Animals*, *12*(7), 832. https://doi.org/10.3390/ani12070832

Kannan, K., & Jain, S. K. (2000). Oxidative stress and apoptosis. *Pathophysiology*, *7*(3), 153-163. https://doi.org/10.1016/S0928-4680(00)00053-5

Karahalil, F. Y., & Şahin, H. (2011). Phenolic composition and antioxidant capacity of cherry laurel (Laurocerasus officinalis Roem.) sampled from Trabzon region, Turkey. *African Journal of Biotechnology*, *10*(72), 16293-16299. https://doi.org/10.5897/AJB11.1929

Kolayli, S., Küçük, M., Duran, C., Candan, F., & Dinçer, B. (2003). Chemical and antioxidant properties of Laurocerasus officinalis Roem.(cherry laurel) fruit grown in the Black Sea region. *Journal of Agricultural and Food Chemistry*, *51*(25), 7489-7494. https://doi.org/10.1021/jf0344486

Kryston, T. B., Georgiev, A. B., Pissis, P., & Georgakilas, A. G. (2011). Role of oxidative stress and DNA damage in human carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *711*(1-2), 193-201. https://doi.org/10.1016/j.mrfmmm.2010.12.016

Loida, P. J., & Sligar, S. G. (1993). Molecular recognition in cytochrome P-450: mechanism for the control of uncoupling reactions. *Biochemistry*, *32*(43), 11530-11538. https://doi.org/10.1021/bi00094a009

Lowry, O. H. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., Núñez, M. a. J., & Parajó, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*(2), 145-171. https://doi.org/10.1016/S0308-8146(00)00223-5

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Orhan, I. E., & Akkol, E. K. (2011). Estimation of neuroprotective effects of Laurocerasus officinalis Roem.(cherry laurel) by in vitro methods. *Food Research International*, *44*(3), 818-822. https://doi.org/10.1016/j.foodres.2011.01.037

Özkara, A., Akyıl, D., & Konuk, M. (2016). *Pesticides, environmental pollution, and health. Environmental Health Risk-Hazardous Factors to Living Species*. Crotia, IntechOpen. https://doi.org/10.5772/63094

Pleban, P. A., Munyani, A., & Beachum, J. (1982). Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. *Clinical Chemistry*, *28*(2), 311-316. https://doi.org/10.1093/clinchem/28.2.311

Pourmorad, F., Hosseinimehr, S., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, *5*(11), 1142-1145.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed Research International*, *2014,* 1-19. https://doi.org/10.1155/2014/761264

Risom, L., Møller, P., & Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *592*(1-2), 119-137. https://doi.org/10.1016/j.mrfmmm.2005.06.012

Saafi-Ben Salah, E. B., El Arem, A., Louedi, M., Saoudi, M., Elfeki, A., Zakhama, A., Najjar, M. F., Hammami, M., & Achour, L. (2012). Antioxidant-rich date palm fruit extract inhibits oxidative stress and nephrotoxicity induced by dimethoate in rat. *Journal of Physiology and Biochemistry*, *68*(1), 47-58. https://doi.org/10.1007/s13105-011-0118-y

Sayim, F. (2007). Dimethoate-induced biochemical and histopathological changes in the liver of rats. *Experimental and Toxicologic Pathology*, *59*(3-4), 237-243. https://doi.org/10.1016/j.etp.2007.05.008

Sharma, Y., Bashir, S., Irshad, M., Gupta, S. D., & Dogra, T. D. (2005a). Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicology*, *206*(1), 49-57. https://doi.org/10.1016/j.tox.2004.06.062

Sharma, Y., Bashir, S., Irshad, M., Nag, T., & Dogra, T. (2005b). Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology*, *215*(3), 173-181. https://doi.org/10.1016/j.tox.2005.06.029

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration*, *82*(2), 291-295. https://doi.org/10.1113/expphysiol.1997.sp004024

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, *175*(1), 184-191. https://doi.org/10.1016/0014-4827(88)90265-0

T.C. TOB. (2022). Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı, Gıda ve Kontrol Genel Müdürlüğü, Aktif Madde Kısıtlama ve Yasaklamaları (Chloridazon, Desmedipham, Dimethoate, Ethoprophos ve Linuron). Retrieved 1.12.2022 from https://bku.tarimorman.gov.tr/Duyuru/DuyuruDetay/81?csrt=5577822346345255811

Yahia, D., & Ali, M. F. (2018). Assessment of neuro-hepatic DNA damage in male Sprague–Dawley rats exposed to organophosphates and pyrethroid insecticides. *Environmental Science and Pollution Research*, *25*(16), 15616-15629. https://doi.org/10.1007/s11356-018-1776-x