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Research Article

Safranal Attenuates Malathion-Induced Oxidative Stress and AChE Inhibition Across Blood, Hepatic, Renal, and Brain Matrices

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ABSTRACT

Organophosphate pesticides, particularly malathion, are widely used in agriculture but pose severe toxicity risks to non-target organisms through acetylcholinesterase (AChE) enzyme inhibition and the induction of oxidative stress. This study investigated the potential protective effects of safranal, a primary bioactive component of saffron (*Crocus sativus* L.) known for its antioxidant and anti-inflammatory properties, against malathion-induced systemic and tissue-specific toxicity. Experimental groups were evaluated by measuring malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels, alongside AChE enzyme activity in the blood, liver, kidney, and brain tissues. The findings demonstrated that malathion exposure caused profound toxicity characterized by severe AChE inhibition, accelerated lipid peroxidation, elevated NO levels, and a critical depletion of both enzymatic and non-enzymatic antioxidant defenses across all analyzed matrices. Conversely, safranal administration served as an effective therapeutic intervention. It mitigated oxidative damage, limited the exhaustion of key antioxidant enzymes by reducing reactive oxygen species, and supported the preservation of AChE functionality. Notably, safranal's protective efficacy exhibited tissue-dependent variations; while it significantly attenuated oxidative stress markers and preserved cellular viability in the plasma, liver, and renal matrices, it demonstrated limited protective capacity in brain tissue under the current experimental conditions. In conclusion, safranal represents a promising bioactive countermeasure against organophosphate-induced systemic injury. However, further research is warranted to elucidate its precise transport mechanisms and potential limitations in crossing the blood-brain barrier.

Keywords: *Acetylcholinesterase, malathion, organophosphates, oxidative stress, safranal*

Safranal kan, karaciğer, böbrek ve beyin matrislerinde malation kaynaklı oksidatif stresi ve AChE inhibisyonunu hafifletir

ÖZET

Başta malation olmak üzere organofosfatlı pestisitler tarımda yaygın olarak kullanılmakta, ancak asetilkolinesteraz (AChE) enzim inhibisyonu ve oksidatif stresi tetikleme mekanizmalarıyla hedef olmayan organizmalar üzerinde ciddi toksisite riskleri oluşturmaktadır. Bu çalışmada, safranın (*Crocus sativus* L.) antioksidan ve anti-enflamatuar özellikleri ile bilinen temel biyoaktif bileşeni safranalın, malation kaynaklı sistemik ve dokuya özgü toksisiteye karşı olası koruyucu etkileri araştırılmıştır. Deneysel gruplar; kan, karaciğer, böbrek ve beyin dokularında malondialdehit (MDA), nitrik oksit (NO), glutatyon (GSH), süperoksit dismütaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPx) düzeylerinin yanı sıra AChE enzim aktivitesi ölçülerek değerlendirilmiştir. Bulgular, malation maruziyetinin; şiddetli AChE inhibisyonu, hızlanmış lipid peroksidasyonu, yükselmiş NO seviyeleri ve analiz edilen tüm matrislerde hem enzimatik hem de enzimatik olmayan antioksidan savunma hatlarında kritik bir tükenme ile karakterize derin bir toksisiteye yol açtığını göstermiştir. Buna karşılık, safranal uygulaması etkili bir terapötik müdahale sağlamıştır. Safranal oksidatif hasarı hafifletmiş, reaktif oksijen türlerini azaltarak temel antioksidan enzimlerin tükenmesini sınırlandırmış ve AChE fonksiyonelliğinin korunmasını desteklemiştir. Özellikle, safranalın koruyucu etkinliği dokuya bağlı varyasyonlar göstermiştir; plazma, karaciğer ve böbrek matrislerinde oksidatif stres belirteçlerini önemli ölçüde azaltıp hücresel canlılığı korurken, mevcut deneysel koşullar altında beyin dokusunda sınırlı bir koruyucu kapasite sergilemiştir. Sonuç olarak safranal, organofosfat kaynaklı sistemik hasara karşı gelecek vaat eden biyoaktif bir önlem olarak öne çıkmaktadır. Bununla birlikte, kan-beyin bariyerini geçmedeki kesin taşınma mekanizmalarını ve olası sınırlılıklarını aydınlatmak için daha fazla araştırmaya ihtiyaç duyulmaktadır.

Anahtar kelimeler: *Asetilkolinesteraz, malathion, oksidatif stres, organofosfatlar, safranal*

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Introduction

Organophosphates (OP) that include compounds such as chlorpyrifos, diazinon, dichlorvos, fenthion, malathion, parathion, soman and sarin are an important pesticide family (Saad et al., 2025). It is believed that most OP compounds exert their effect via similar metabolic pathways. After several OP compounds, most of which are highly soluble in fat, are absorbed through the skin, the conjunctiva and gastrointestinal tract or inhaled through the respiratory tract, they are rapidly metabolized and transformed into oxonium forms, inhibiting the AChE enzyme (Barr and Angerer, 2006). Thus, they are not hydrolyzed during acetylcholine neurotransmission but accumulate in all ganglia in the autonomous nervous system, several synapses in brain and skeletal muscle neuromuscular junctions and certain postganglionic nerve endings of the sympathetic nervous system (Chen et al., 2024). However, it was also reported that the OP compounds had adverse effects on several tissues such as liver, lung, heart, kidney, jejunum and pancreas (Buyukokuroglu et al., 2008) and bio-element levels (Cemek et al., 2010a).

It was also reported that OP compounds increase the free radical production and inhibit the activity of antioxidant enzymes such as SOD, GPx and CAT, thereby destroying the balance between oxidants and antioxidants and causing oxidative stress (Cemek et al., 2010b).

The transformation of malathion, a phosphorodithioate type OP compound, which is commonly used as an insecticide in agriculture, into malaoxon, which is a 60 times more toxic compound when compared to malathion, in time demonstrates that it should be used with care (Lasram et al., 2014a). Malathion was reported to be associated with conditions such as cardiotoxicity, hepatotoxicity, neurotoxicity, inflammation and cytotoxicity (Han and Wen, 2026), although irreversible binding of malathion with AChE results in the elimination of its enzyme activity. The fact that malathion causes oxidative DNA damage, lipid peroxidation and oxidative protein damage (Ullah et al., 2018) suggests that this compound is also associated with oxidative stress. Decrease in antioxidant enzyme activities (Durak et al., 2009) such as SOD, GPx and CAT as well as the increase in total oxidant level (Basarslan et al., 2014) are demonstrated as the main causes of malathion-induced oxidative stress.

Safranal, used both in the kitchen and for alternative medicine in oriental societies, is a compound that provides its scent to saffron (Butnariu et al., 2022). Safranal was reported to have several pharmacological activities. Safranal was reported to have several pharmacological activities, including anti-inflammatory (Tamaddonfard et al., 2013) and antioxidant properties (Samarghandian et al., 2013).

The central hypothesis of this study is that safranal has the potential to protect against malathion-induced tissue damage by counteracting oxidative stress and alleviating AChE enzyme dysfunction. To this end, in the present study, possible protective effects of safranal on malathion toxicity were investigated. MDA, NO, GSH, SOD, CAT, GPx levels and AChE enzyme activity were measured in blood and various tissues (liver, kidney and brain) in all study groups.

Materials and Methods

Chemicals

Malathion was procured as a commercial emulsifiable concentrate (Malathion 65 EM, Agrofarm Corporation, Istanbul, Turkey), which contains 65% active malathion ingredient and safranal (Safranal ≥88%) was obtained from Sigma-Aldrich (Darmstadt, Germany). Sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, butylated hydroxytoluene, trichloroacetic acid, EDTA, thiobarbituric acid, sodium hydroxide, glutathione standard, metaphosphoric acid [5,5-dithiobis- (2-nitrobenzoic acid)] (DTNB), sodium citrate, Potassium dihydrogen phosphate, hydrogen peroxide, vanadium (III) chloride, sulfanilamide, N-(1-Naphthyl) ethylenediamine dihydrochloride were obtained from Merck (Darmstadt, Germany), commercial kits for SOD and GPx analyses (RANSOD and RANSEL) were procured from Randox Corporation (Crumlin, County Antrim, Northern Ireland, UK). Malathion was diluted in corn oil, and safranal was diluted in physiologic saline solution. Ultra-pure water was used as solvent.

Animals

A cohort of 42 male Wistar rats (age range: 6–8 weeks; weight spectrum: 210–300 g) was utilized for the *in vivo* phase. The animals were housed under strictly monitored environmental baselines, maintained at a stable ambient temperature of 24±2°C and a relative humidity range of 55–60%, under a structured 12-hour light/dark photoperiod. The rats had unrestricted, ad libitum access to standardized dry pellet food and daily replenished fresh water. To eliminate transport-induced stress, a baseline acclimatization phase of 7 days was enforced prior to any experimental intervention. This study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee (decision number: B.30.2.AKÜ.0.A2.00.00/249, date: 09.11.2012).

Experimental Design

Forty-two rats were allocated into six experimental groups (n=7 per group) using a weight-stratified block randomization procedure to ensure equivalent baseline body weight distributions across all cohorts. To minimize environmental confounders and ensure cage balancing, animals from different experimental groups were co-housed where feasible, and all cages were subjected to identical handling protocols. The experimental groups and the rationale behind the vehicle administrations were designed as follows:

- Control group (CONTROL): The rats in this group

were included in the study without administering any agent or vehicle, serving as an untreated baseline to monitor physiological and biochemical parameters under standard laboratory conditions.

- Sham group (SHAM): To serve as a precise volume-, vehicle-, and route-matched control, the rats in this group were administered corn oil subcutaneously (s.c.) as the vehicle control for malathion, and physiologic saline solution intraperitoneally (i.p.) as the vehicle control for safranal. This dual vehicle administration ensured that any technical stress or vehicle-induced effect was precisely controlled.
- Malathion group (MAL): The rats were injected with a single dose of 250 mg/kg malathion s.c.
- Safranal group (SAF): The rats were administered 200 mg/kg safranal i.p.
- Safranal + Malathion group (SAF+MAL): To evaluate the protective/preventive effect, 200 mg/kg safranal was administered via i.p., followed by the s.c. injection of 250 mg/kg malathion 1 hour later.
- Malathion + Safranal group (MAL+SAF): To evaluate the therapeutic effect, 250 mg/kg malathion was injected s.c., and 200 mg/kg safranal was applied via i.p. 1 hour later.

The malathion dose (250 mg/kg) was chosen to induce robust acute oxidative stress and AChE inhibition without immediate lethality (1/12 LD_{50}) (Contreras and Bustos-Obregon, 1999) while the subcutaneous route was utilized to simulate acute exposure. To administer a precise dose of 250 mg/kg of the active malathion, the required volume of the commercial formulation was calculated based on this 65% active concentration and subsequently diluted in corn oil. Conversely, the safranal dose (200 mg/kg) (Sadeghnia et al., 2013) and its intraperitoneal administration were selected to ensure rapid systemic bioavailability and peak antioxidant efficacy. Safranal was diluted in physiologic saline solution The 1-hour pre- and post-exposure intervals were specifically designed to evaluate the immediate prophylactic and therapeutic capacities of safranal relative to the rapid onset of organophosphate-induced biochemical alterations.

The 24-hour endpoint was designated to assess acute tissue-specific toxicity and early-stage oxidative damage. 24 hours after the administrations, the experimental period was concluded. Blood and tissue samples (liver, kidney, and brain) were collected from all animals under deep ketamine/xylazine anesthesia for subsequent biochemical analyses.

Biochemical Analyses

Blood samples collected into heparinized tubes were centrifuged at 3000 rpm for 15 minutes at +4°C to separate the plasma, which was then transferred into distinct Eppendorf tubes. The remaining erythrocytes were washed three times with 0.9% saline solution and analyzed on the same day. Liver, kidney, and brain tissues were homogenized in a 10-fold volume of physiological saline solution using a tissue homogenizer. The resulting homogenates were centrifuged at 10000 rpm for 1 hour to obtain clear supernatants, which were subsequently analyzed within the same day. MDA is one of the final products generated by free radicals via the peroxidation of unsaturated fatty acids. MDA reacts with thiobarbituric acid to form a colored complex. Lipid peroxidation levels in plasma and tissue samples were determined spectrophotometrically based on the absorbance of this colored complex at wavelengths of 532 nm and 600 nm (Jain et al., 1989). To determine GSH levels, all sulfhydryl group-containing proteins in the erythrocytes and tissue supernatants were precipitated using a precipitation solution and removed by filtration. GSH content was determined by measuring the absorbance of the yellow-colored product formed upon reaction with 5,5-dithiobis-(2-nitrobenzoic acid) in the clear filtrate at 412 nm (Beutler et al., 1963). NO content in the plasma and tissues was determined spectrophotometrically using the VCl_3/Griess method (Miranda et al., 2001). Commercial kits were utilized to determine SOD and GPx enzyme activities in the erythrocytes. Tissue and erythrocyte catalase CAT activities were measured spectrophotometrically by monitoring the hydrogen peroxide decomposition reaction. A rat-specific ELISA kit (Cusabio Chemical Company, Wuhan, Hubei Province, PR China) was employed to measure erythrocyte AChE activity.

To prevent measurement and observer bias, the investigators responsible for tissue processing, executing the biochemical assays, and conducting the statistical analyses were completely blinded to the experimental group allocations. The samples were coded, and unblinding occurred only after the final data set was independently generated and statistically evaluated.

Statistical Analyses

Statistical analyses of the obtained findings were conducted using SPSS 17.0 software. The data were expressed as mean ± standard deviation (mean ± SD). Prior to parametric tests, the distribution of data was assessed for normality using the Shapiro–Wilk test, and the homogeneity of variances was verified using Levene's test. Data that met the parametric assumptions were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test to determine statistical differences between the groups. For all statistical analyses, the significance level was set at $p < 0.05$.

Results

Significant differences ($p < 0.05$) were determined between Control and Sham groups and Malathion, Safranal, Safranal + Malathion and Malathion + Safranal groups ($p < 0.05$), while there was no difference between Control and Sham groups based on plasma MDA levels. It was also found that MDA level decreased in Safranal + Malathion and Malathion + Safranal groups compared to

malathion group, but the differences were not significant (Figure 1A). The NO level was the lowest in the Safranal group and it was significantly different from the Control group ($p < 0.05$), while no statistical difference was observed between the other groups (Figure 1B).

A significant increase was observed in the erythrocyte GSH levels of all safranal administered groups when compared to Control, Sham and Malathion groups ($p < 0.05$), while there was a significant difference between erythrocyte GSH levels in Sham and Malathion groups (p

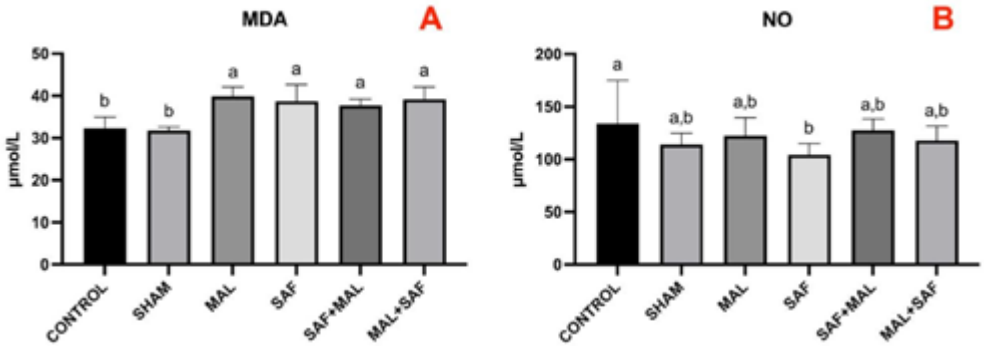

Figure 1. MDA (A) and NO (B) concentrations in plasma

[a,b] Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are presented as mean ± SD (n=7). MDA: Malondialdehyde, NO: Nitric oxide; MAL: Malathion group; SAF: Safranal group; SAF+MAL: Safranal+Malathion group: MAL+SAF: Malathion+Safranal group.A: Plasma MDA levels; B: Plasma NO levels

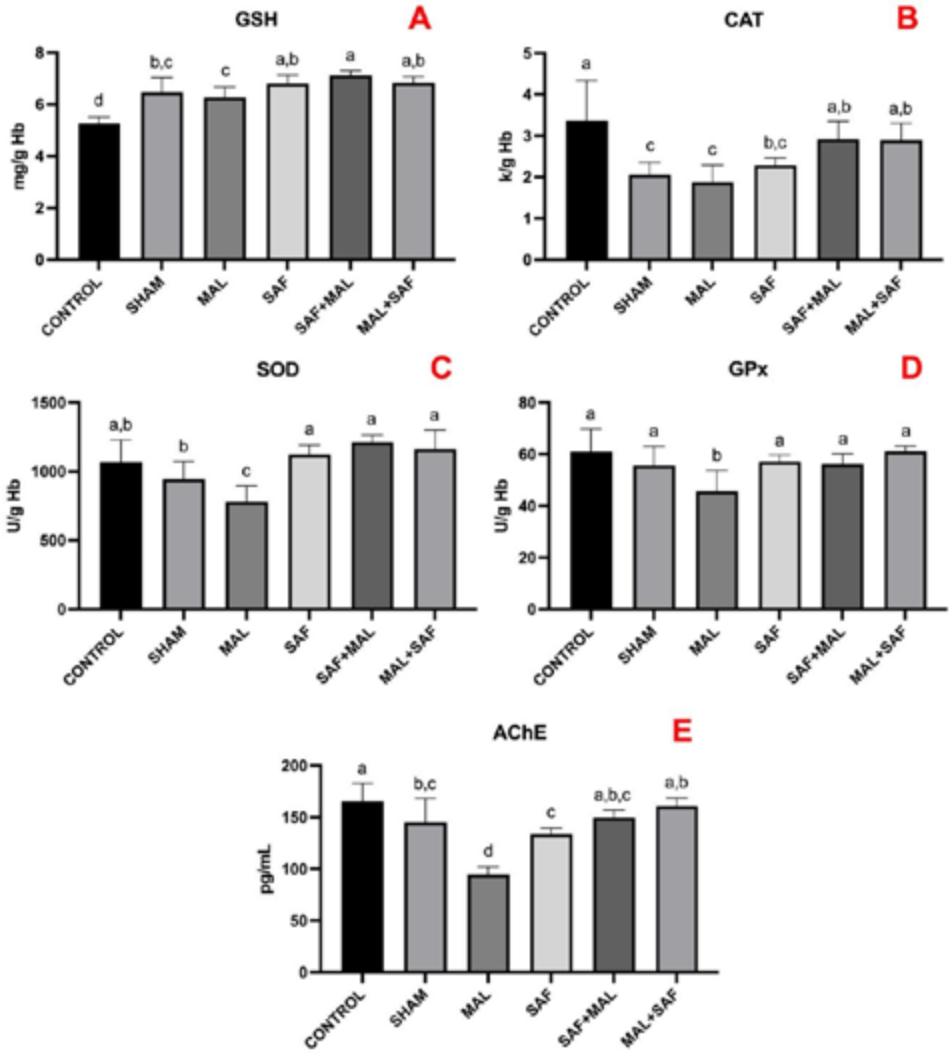

Figure 2. GSH (A), CAT (B), SOD (C), GPx (D) and AChE (E) concentrations in erythrocytes

[a,b,c,d] Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are presented as mean ± SD (n=7). GSH: Reduced glutathione, CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; AChE: Acetylcholinesterase; MAL: Malathion group; SAF: Safranal group; SAF+MAL: Safranal+Malathion group: MAL+SAF: Malathion+Safranal group.
A: Erythrocyte GSH levels; B: Erythrocyte CAT levels; C: Erythrocyte SOD levels; D: Erythrocyte GPx levels; E: Erythrocyte AChE levels

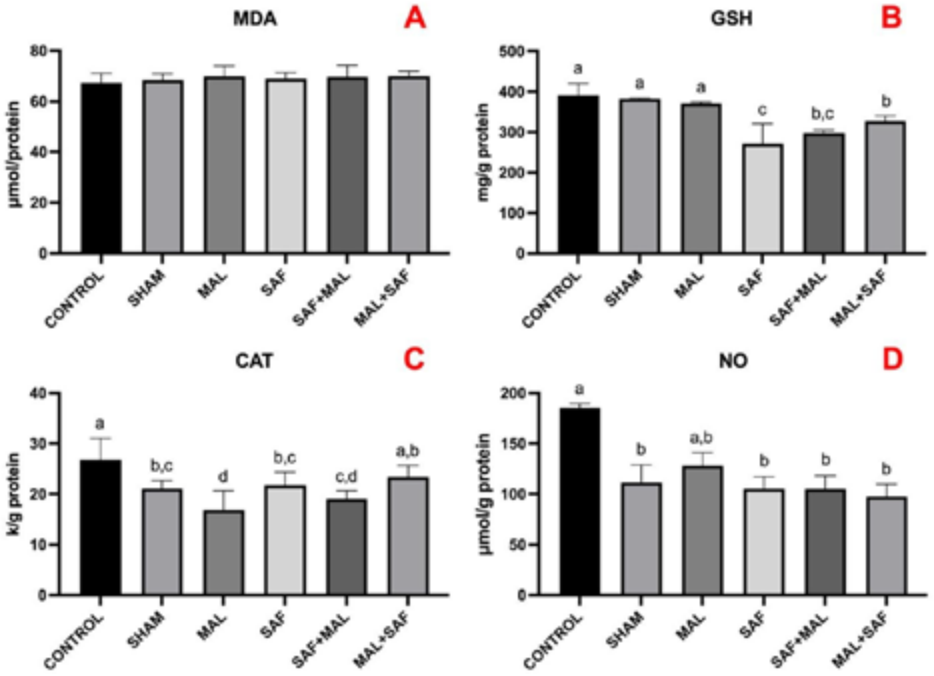

Figure 3. MDA (A), GSH (B), CAT (C) and NO (D) concentrations in liver
[a,b,c,d] Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are presented as mean ± SD (n=7). MDA: Malondialdehyde; GSH: Reduced glutathione, CAT: Catalase; NO: Nitric oxide; MAL: Malathion group; SAF: Safranal group; SAF+MAL: Safranal+Malathion group: MAL+SAF: Malathion+Safranal group. A: Liver MDA levels; B: Liver GSH levels; C: Liver CAT levels; D: Liver NO levels

<0.05) (Figure 2A). It was also found that erythrocyte CAT levels in treatment groups of Safranal + Malathion and Malathion + Safranal groups were higher than the Sham and Malathion groups ($p < 0.05$), and the erythrocyte CAT levels of the Control group were statistically different from those of the Sham, Malathion and Safranal groups (Figure 2B). The erythrocyte SOD activities in all safranal injected groups increased significantly ($P < 0.05$) when compared to Sham and Malathion groups, and the lowest erythrocyte SOD activity was detected in the Malathion group (Figure 2C). Erythrocyte GPx activity was the lowest in Malathion group and it was statistically different from all other groups ($p < 0.05$) (Figure 2D). Also, the erythrocyte AChE enzyme activity was the lowest in the Malathion group, and it was determined that the safranal administration significantly increased AChE activity in all groups (Figure 2E).

Although there was no statistical difference between the liver MDA levels in all groups (Figure 3A), there was a significant decrease in GSH levels in safranal administered groups when compared to Control, Sham and Malathion groups ($p < 0.05$). Furthermore, a significant difference was found between the hepatic GSH levels in the Malathion + Safranal and the Safranal groups ($p < 0.05$) (Figure 3B). When compared to the Malathion group where the lowest CAT activity was observed, it was determined that the CAT levels in the Safranal and Malathion + Safranal groups increased statistically significantly ($p < 0.05$) (Figure 3C). NO levels in the Sham, Safranal, Malathion and Malathion + Safranal groups decreased significantly when compared to the liver NO levels in the Control group ($p < 0.05$). A similar decrease was found in all safranal administered groups when compared to the Malathion group but the difference was not significant (Figure 3D).

It was determined that kidney MDA levels in Safranal, Safranal + Malathion and Malathion + Safranal groups were lower when compared to Control, Sham and Malathion groups, but MDA levels were statistically significantly lower in only Safranal and Malathion + Safranal groups ($p < 0.05$) (Figure 4A). Kidney GSH levels were significantly lower in Malathion and Control groups when compared to the Safranal + Malathion group but higher in the Safranal and Malathion + Safranal group ($p < 0.05$) (Figure 4B). There was a statistically significant increase in kidney CAT activities between the Safranal, Safranal + Malathion and Malathion + Safranal groups when compared to the Malathion group ($p < 0.05$), while there was no difference between kidney CAT activities between the Control, Sham and Malathion groups (Figure 4C). In Safranal, Malathion + Safranal groups and the Safranal + Malathion group with the highest kidney NO levels, it was determined that kidney NO levels increased when compared to Malathion, Sham and Control groups. Differences in renal NO levels were observed especially between Safranal + Malathion and Malathion + Safranal groups and others ($p < 0.05$) (Figure 4D).

It was determined that brain MDA levels in Control and Sham groups were significantly higher than the other four groups ($p < 0.05$). There were no significant differences between the Malathion group and all safranal administered groups (Figure 5A). It was identified that brain GSH levels were significantly lower in Safranal, Safranal + Malathion, and Malathion + Safranal groups when compared to that of the Malathion group with the

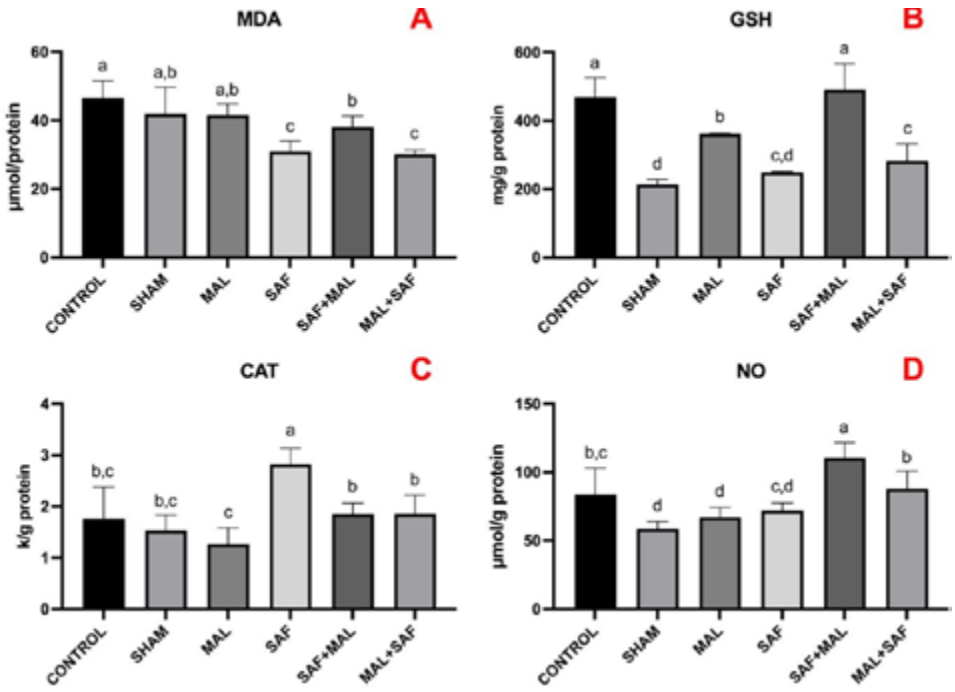

Figure 4. MDA (A), GSH (B), CAT (C) and NO (D) concentrations in kidney
[a,b,c,d] Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are presented as mean ± SD (n=7). MDA: Malondialdehyde; GSH: Reduced glutathione, CAT: Catalase; NO: Nitric oxide; MAL: Malathion group; SAF: Safranal group; SAF+MAL: Safranal+Malathion group: MAL+SAF: Malathion+Safranal group.
A: Kidney MDA levels; B: Kidney GSH levels; C: Kidney CAT levels; D: Kidney NO levels

highest brain GSH level ($p < 0.05$) (Figure 5B). While there were no significant differences between the brain CAT activities in Malathion, Safranal, Safranal + Malathion, and Malathion + Safranal groups, it was determined that brain CAT activities were significantly lower in these groups when compared to Control and Sham groups ($p < 0.05$) (Figure 5C). The Safranal group demonstrated the lowest brain NO levels and these values were different from all other groups, while the brain NO level in Malathion group was significantly lower when compared to the Control group ($p < 0.05$) (Figure 5D).

Discussion

OP compounds are the most widely used chemicals in the world despite their high toxicity. They are easily accessible and their intense and unconscious use causes major health problems, environmental pollution and economic losses in several countries (Cemek et al., 2010b). These

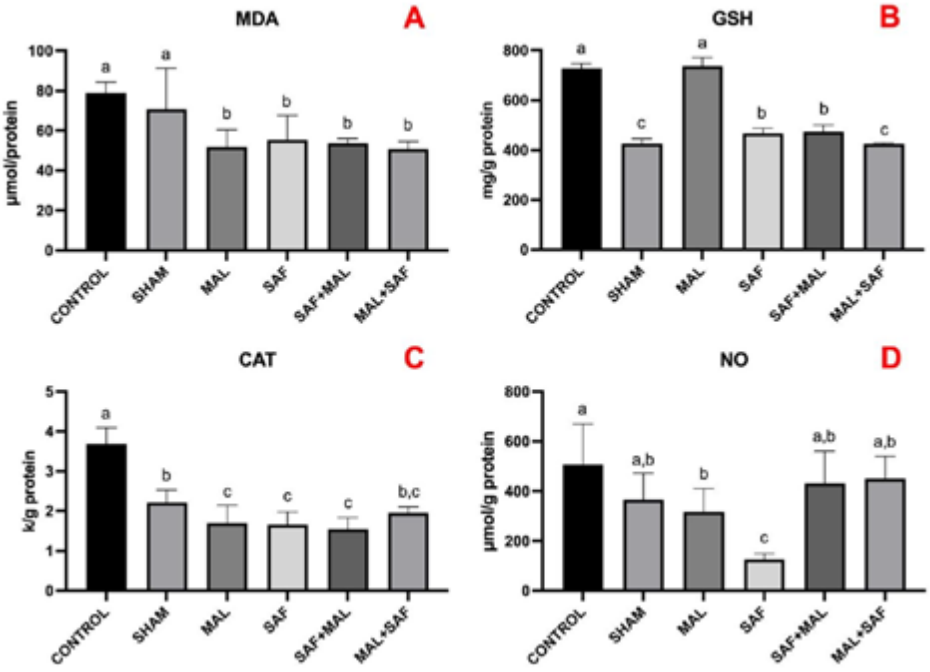

Figure 5. MDA (A), GSH (B), CAT (C) and NO (D) concentrations in brain
[a,b,c] Different superscript letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Values are presented as mean ± SD (n=7). MDA: Malondialdehyde; GSH: Reduced glutathione, CAT: Catalase; NO: Nitric oxide; MAL: Malathion group; SAF: Safranal group; SAF+MAL: Safranal+Malathion group: MAL+SAF: Malathion+Safranal group. A: Brain MDA levels; B: Brain GSH levels; C: Brain CAT levels; D: Brain NO levels

compounds that include malathion generally act by inhibiting the AChE enzyme, which is responsible for the rapid hydrolysis of acetylcholine neurotransmitter during neurotransmission in cholinergic synapses (Aroniadou-Anderjaska et al., 2023). In a study that investigated the protective role of N-acetyl-L-cysteine against malathion toxicity, it was determined that AChE levels decreased in the malathion administered group when compared to the control (Lasram et al., 2014b), in another study where the effects of malathion on glucose homeostasis were investigated, it was found that malathion decreased AChE activity (Mostafalou et al., 2012) and blood AChE levels of employees that were exposed to OP pesticides were reported to be lower when compared to the control group (Singh et al., 2011). Consistent with the abovementioned studies, the fact that the erythrocyte AChE levels in the malathion group was lower than that of the control, sham and safranal groups in the present study is consistent with the reduction in free AChE levels due to the irreversible binding of the malathion to the AChE enzyme in the synaptic space. Furthermore, it was considered that safranal administration suppressed the high stimulation by increasing the AChE enzyme levels, thus, spontaneously accelerating the slow enzyme regeneration, leading to an increase in free AChE levels.

Another toxic effect of OP compounds except inhibiting the AChE enzyme is the production of the destructive molecules called reactive oxygen species (ROS) or facilitating the adverse effects of these molecules on lipids DNA-RNA, or proteins (Mostafalou et al., 2012). Both *in vivo* and *in vitro* studies demonstrated the action of OP compounds in the production of ROS (Aboubakr et al., 2019).

Lipid peroxidation is a naturally occurring process in the metabolism due to several ROS. MDA, which is produced in the last step of peroxidation has a good correlation with the degree of damage, is utilized to measure the level of lipid peroxidation that occurs in the organism (Draper et al., 2019). In several previous studies, it was determined that the administration of OP compounds increased lipid peroxidation (Buyukokuroglu et al., 2008; Cemek et al., 2010a; Cemek et al., 2010b) and lipid peroxidation was inhibited by the impact of antioxidants such as crocin (Dorri et al., 2015). Consistent with this data, it was determined in the present study that malathion administration triggered lipid peroxidation and increased MDA levels in the plasma, and kidney tissues, and in safranal administered groups, lipid peroxidation was broken due to the antioxidant scavenging effect of safranal in the plasma and renal and liver tissues when compared to malathion groups, leading to a reduction in MDA levels. It was determined that there was no protective effect of safranal based on the MDA levels in brain tissue.

Increased concentration of endogenous nitric oxide (NO) in the metabolism, which consists of nitric oxide synthase (NOS) and the amino acid L-arginine, plays an important role in the formation of oxidative stress and tissue damage (Janaszak-Jasiecka et al., 2023). Although increased NO levels were reported in organisms exposed to organophosphates (Karabag-Coban et al., 2016), it was determined that administration of safranal caused a decrease in NO levels (Lertnimitphun et al., 2019). In the present study, it was observed that plasma, kidney and brain NO levels increased in association with the malathion administration induced tissue damage when compared to the sham group, while plasma and liver NO levels decreased in the safranal administered groups, although the difference was not significant.

Reduced GSH is a non-enzymatic antioxidant with significant roles in the detoxification of and cellular defense against reactive products, oxygen radicals and toxic aldehydes. In the case of oxidative stress, reactive oxygen species are reduced by GSH, while GSH is oxidized to GSSG (Oxide glutathione) (T.-H. Chen et al., 2024). It was reported that malathion administration reduced GSH levels in blood, liver, kidney and brain tissues, as well as the oxidative stress (Ramadan et al., 2017). In certain previous studies, it was observed that safranal facilitated the reduction of reactive species to less harmful species by increasing GSH concentration (Samarghandian et al., 2013; Samarghandian et al., 2015; T.-H. Chen et al., 2024). In the present study, the increase in erythrocyte GSH levels with safranal administration was an indicator of antioxidant response in the blood. On the other hand, it was reported that safranal administration against oxidative stress reduced GSH levels (Hazman and Bozkurt, 2015) and similar findings were observed in studies where several antioxidants were administered against organophosphate toxicity (Cemek et al., 2010a; Ramadan et al., 2017). It was reported that this reduction resulted in overproduction of of hydrogen peroxide reactive oxygen species and the increase in the consumed GSH amounts along with extreme increase in these species would reduce the overall GSH levels. Based on the information provided above, it was determined in the present study that especially the brain and kidney GSH levels were affected by oxidative stress more than the blood tissue based on the MDA and NO levels and consistent with this fact, GSH levels decreased in safranal administered groups.

In addition to non-enzymatic defense systems including GSH, enzymatic defense systems including CAT, SOD and GPx enzymes are also effective in the detoxification of reactive oxygen species (Ighodaro and Akinloye, 2018).

In previous studies, it was reported that malathion decreased blood, liver, kidney and brain tissue CAT activities, SOD antioxidant enzyme levels (Akbel et al., 2018) and GPx levels. Similarly, in the present study, significant reductions in enzyme levels were observed in malathion administered groups for all tissues when compared to the control group. This was due to the intensive spending of antioxidant enzymes to inhibit organophosphate toxicity induced ROS production. It was reported that safranal increased CAT, SOD and GPx enzyme activation against hyperglycemia, hyperlipidemia

and oxidative stress in blood tissues (Samarghandian et al., 2013), also safranal administration caused an increase in GPx, SOD and CAT levels (Cerdá-Bernad et al., 2022). Furthermore, it was observed that safranal also increased SOD levels in the brain tissues with oxidative damage (Samarghandian et al., 2015). In the present study, safranal reduced the oxidative stress by reducing the presence of reactive oxygen species in erythrocyte, liver and renal tissues in treatment groups, and concurrently, an increase in enzyme activities was observed due to the limited expense of the CAT, SOD and GPx enzymes.

Despite the significant findings regarding the protective and therapeutic effects of safranal against malathion-induced systemic toxicity, this study has several limitations that should be acknowledged. First, the experimental design utilized exclusively male Wistar rats, which precludes the assessment of potential sex-dependent differences in toxicological and neuroprotective responses. Second, the evaluations were based on a single-dose regimen and a specific 24-hour endpoint. Because our findings reflect one acute time point rather than capturing the full biphasic toxicodynamics or peak kinetic effects of malathion and safranal, comprehensive dose-response relationships could not be established. This temporal limitation is particularly crucial for interpreting our tissue-specific findings; it may explain why certain endpoints, notably MDA and NO levels in the brain and liver matrices, did not follow expected canonical patterns at 24 hours. The peak oxidative burst or the subsequent antioxidant compensatory mechanisms in these specific tissues might occur either earlier or later than our selected evaluation window. Furthermore, the use of different administration routes—subcutaneous for malathion and intraperitoneal for safranal—may introduce variations in absorption kinetics and systemic bioavailability. Moreover, the use of a commercial formulation (Malathion 65 EM) introduces a potential confounding factor, as undisclosed proprietary co-formulants may independently influence redox biology, inflammation, or cellular membranes. Consequently, while our SHAM group (corn oil) precisely controlled for the dilution vehicle, it could not account for the potential baseline biochemical effects of the full commercial matrix. Additionally, the assessment of tissue damage and safranal's protective efficacy relied entirely on biochemical markers and antioxidant enzyme activities. The lack of histopathological verification of tissue architecture and molecular analyses of specific gene expressions restricts a complete elucidation of the underlying mechanisms. Finally, while safranal demonstrated robust protective efficacy in reducing oxidative stress in plasma, hepatic, and renal matrices, its capacity in brain tissue was notably limited. This discrepancy strongly suggests potential constraints in safranal's ability to effectively cross the blood-brain barrier under the current experimental conditions. Future studies incorporating varied dosage regimens, diverse time points, histological validations, and both sexes are warranted to fully clarify safranal's systemic and central nervous system efficacy.

Conclusion

In conclusion, safranal demonstrates significant protective potential against malathion-induced systemic toxicity by preserving antioxidant defense systems and mitigating AChE inhibition, particularly in peripheral matrices such as blood, liver, and kidneys. However, its limited efficacy in brain tissue suggests potential constraints regarding blood-brain barrier permeability under the current experimental conditions. These findings position safranal as a promising bioactive agent for counteracting organophosphate exposure. Future research is warranted to fully elucidate its pharmacokinetic profile, administration route efficiency, and central nervous system accessibility.

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Author contribution statement

Conceptualization, methodology, resources, original draft preparation, review and editing: A.B; data analysis: O.H; data curation: L.A; investigation: A.B, O.H, L.A, F.K. The approach used to determine the author order was strictly based on the extent, volume, and significance of each individual's scientific contribution to the experimental design, data acquisition, and manuscript preparation. All authors have read and explicitly approved the final version of the manuscript for submission.

Conflict of Interest

The authors declared that we have no conflicts of interest to this work.

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