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RESEARCH ARTICLE

The Protective Effects of *Nigella Sativa* on the Antioxidant System and Certain Cytokine Levels in Rats Exposed to Experimental Acrylamide

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Nigella sativa'nın Deneysel Akrilamid Uygulanan Ratlarda Antioksidan Sistem ve Bazı Sitokin Düzeyleri Üzerindeki Koruyucu Etkisi

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Öz

Amaç: Bu çalışmanın amacı, *Nigella Sativa*'nın (*N. sativa*) akrilamidin genotoksik, kanserojen ve nörotoksik etkilerine karşı potansiyel koruyucu özelliklerini araştırmaktır. *N. sativa,* çevresel toksinlerin zararlı etkilerine karşı koyabilen güçlü antioksidan ve anti-inflamatuar özellikleri ile bilinir. Araştırmada, *N. sativa*'nın antioksidan sistem ve proinflamatuar sitokinler üzerindeki etkisi ve çeşitli sistemlere zararlı etkileri bilinen bir madde olan akrilamid'e maruz kalan sıçanlarda terapötik potansiyeli araştırılmıştır.

Gereç ve Yöntem: Toplamda 32 sağlıklı yetişkin erkek sıçan kullanıldı ve dört ayrı gruba ayrıldı: kontrol, akrilamid, *N. sativa* ve akrilamid + *N. sativa* grupları. Akrilamid (40 mg/kg/gün) ve *N. sativa* yağı (10 mg/kg/gün) 15 gün boyunca ağızdan verildi. IL-1, IL-6, IL-10, SOD, glutatyon, malondialdehid ve TNF-α seviyeleri analiz edildi.

Bulgular: Akrilamid grubunda, diğer gruplara kıyasla MDA seviyelerinde oksidatif stresi gösteren önemli bir artış gözlendi ($P < 0.05$). Ayrıca, GSH ve SOD seviyeleri akrilamid grubunda anlamlı derecede düşüktü ($P < 0.05$). Buna karşılık, *N. sativa* ile tedavi edilen deneysel grup, akrilamid grubuna kıyasla malondialdehid seviyelerinde yaklaşık %30 azalma ve glutatyon ve süperoksit dismutaz seviyelerinde sırasıyla yaklaşık %40 ve %60 iyileşme gösterdi, bu da akrilamid toksisitesine karşı önemli bir koruyucu etkiyi işaret ediyordu ($P < 0.05$). Akrilamid grubunda ayrıca pro-inflamatuar sitokinlerin (TNF-α, IL-6, IL-1) yükselmiş seviyelerini ve anti-inflamatuar sitokin (IL-10) seviyelerinin düşük olduğu gözlendi ($P < 0.05$). *N. sativa*'nın antienflamatuvar etkisinin bir göstergesi olarak *N. sativa* grubunda, TNF-α, IL-6 ve IL-1 seviyeleri anlamlı derecede azaldı ($P < 0.05$).

Öneri: Çalışma bulguları *N. sativa*'nın akrilamidin neden olduğu oksidatif stres ve inflamasyonu hafifletebileceğini göstermektedir. *N sativa*'nın, ACR tarafından değiştirilen MDA seviyelerindeki artışı önemli ölçüde iyileştirdiği ve antioksidan seviyelerini artırdığı ve ACR tarafından indüklenen IL-1 seviyelerindeki değişiklikleri kısmen hafiflettiği görülmektedir. Bu sonuçlar, *N. sativa*'nın akrilamidin zararlı etkilerini hafifletmede potansiyel faydaları olabileceğini öne sürmektedir.

Anahtar kelimeler: Akrilamid, İnflamasyon, *N. sativa*, Oksidatif Stres, Sitokinler

Abstract

Aim: The objective of this research was to examine the potential safeguarding properties of *Nigella sativa* (*N. sativa*) against the genotoxic, carcinogenic, and neurotoxic effects of acrylamide. *N. sativa* is known for its strong antioxidant and anti-inflammatory characteristics which can counteract the detrimental impact of environmental toxins. The research investigated the influence of N. sativa on the antioxidant system and pro-inflammatory cytokines, and explored its therapeutic potential in rats exposed to acrylamide, a substance known for its harmful effects on various systems.

Materials and Methods: Thirty-two healthy adult male rats were used and allocated into four separate groups: control, acrylamide, *N. sativa*, and acrylamide + *N. sativa* groups. Acrylamide (40 mg/kg/day) and *N. sativa* oil (10 mg/kg/day) were given orally for 15 days. IL-1, IL-6, IL-10, SOD, glutathione, malondialdehyde, and TNF-α levels were analyzed.

Results: The acrylamide group exhibited a significant increase in MDA levels in comparison to the other groups, indicating oxidative stress ($P < 0.05$). Moreover, GSH and SOD levels were appreciably lower in the acrylamide group ($P < 0.05$). Conversely, the experimental group treated with N. sativa displayed reduced MDA levels (by approximately 30%) and improved GSH and SOD levels compared to the acrylamide group, suggesting a significant protective effect against acrylamide toxicity ($P < 0.05$). The acrylamide group showed significantly increased levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1, along with a notable decrease in the anti-inflammatory cytokine IL-10 ($P < 0.05$). Conversely, in the group treated with *N. sativa*, there was a significant reduction in the concentrations of TNF-α, IL-6 and IL-1 ($P < 0.05$), indicating the anti-inflammatory effects of *N. sativa.*

Conclusion: The study results suggest that *N. sativa* can alleviate acrylamide-induced oxidative stress and inflammation. It seemed that *N. sativa* significiantly improved the increase of MDA levels altered by ACR and increase antioxidant levels, and partially attenuate the changes in L-1 levels induced by ACR. These findings indicate that *N. sativa* suggests potential benefits in alleviating the adverse impacts of acrylamide.

Keywords: Acrylamide, Cytokines, Inflammation, *N.sativa*, Oxidative Stress

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Introduction

Acrylamide (ACR) is a chemical substance from which polyacrylamide is produced and used to treat drinking and industrial wastewater. ACR, known as one of the highly effective neurotoxic agents, is used as an additive in industries such as paint manufacturing, fiber processing, polymer production, gel electrophoresis, water treatment, textile, mining, paper production, and cosmetics industry (Uludağ 2017, İnce et al 2018, Guo et al 2020).

Furthermore, diet is also recognized as a major pathway for ACR exposure due to its occurrence in carbohydrate-rich foods subjected to high-temperature cooking methods (Mehri et al 2015). Research has indicated that acrylamide is present in various foods including potato chips, toast, cookies, breakfast cereals, and coffee (Tareke et al 2002). Formed through the Maillard reaction, acrylamide emerges when asparagine and glucose interact at temperatures more than 120°C (Mottram et al 2002). Despite the global concern about acrylamide in foods, its toxicity related to dietary intake has not been experimentally elucidated (İnce et al 2018).

There is evidence that the development of numerous diseases is linked to an imbalance between oxidants and antioxidants, influenced by exposure to harmful substances like acrylamide (Amirshahrokhi 2021). Research suggests that ACR can lead to toxicity in multiple organs by triggering oxidative stress and inflammation (Akkaya et al 2021). Research has found that exposure to ACR leads to a reduction in antioxidant enzymes like SOD, catalase, and GSH across various tissues, causing lipid and protein oxidation (Zamani et al 2018, Tabeshpour et al 2020, Amirshahrokhi 2021).

ACR, a highly toxic substance with possible cancer-causing effects, can penetrate the skin, ingested through the digestive system, and inhaled via respiratory routes. Once inside, it rapidly distributes to different tissues and undergoes metabolism to create glycidamide, which is a highly reactive epoxy compound (Zödl et al 2007, Matoso et al 2019). ACR has been discovered to impact cells through multiple mechanisms, such as inducing inflammation, causing oxidative stress, initiating programmed cell death (apoptosis), and inflicting damage to DNA (Hatipoglu et al 2024). Studies have indicated that ACR exposure leads to increased concentrations of inflammatory cytokines. Specifically, these include significant rises in Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), and Interleukin-6 (IL-6), highlighting the toxic effects of acrylamide. This elevation underscores the substantial inflammatory response elicited by ACR-induced toxicity. These findings emphasize the pronounced inflammatory response elicited by exposure to this harmful compound (Amirshahrokhi 2021).

Natural antioxidants have been the main target of most studies to determine the most effective protective agents to reduce or prevent the adverse effects of ACR toxicity. Within this framework, research has shown that numerous renowned antioxidants, including quercetin, curcumin, crocin, linalool, and chrysin, are effective in combating acrylamide-induced neurotoxicity (Mehri et al 2015). Extensively utilized around the world, N. sativa is employed in treating numerous diseases and as a preventative food additive (Karimi et al 2019).

N. sativa, a dicotyledonous plant from the Ranunculaceae family, is widely recognized under the common names of black seed or black cumin. This plant is known for its distinctive small, black seeds that have been used traditionally for their medicinal properties. This plant, which originates from the regions of South and Southwest Asia, has been widely cultivated and is now found extensively across the Middle East, North Africa, and Southern Europe (Ali and Blunden 2003, Meral et al 2004, Güzelsoy et al 2018). Research has demonstrated that *N. sativa* harbors numerous health-promoting properties. These include its potent antioxidant capabilities, its ability to inhibit tumor growth, its significant anti-inflammatory effects, and its antibacterial properties. Collectively, these attributes underscore the plant's broad therapeutic potential and its traditional use in various medicinal practices. Additionally, it has a stimulating effect on the immune system and demonstrates therapeutic effects against numerous diseases (Asal Ulus et al 2018, Güzelsoy et al 2018).

The numerous health benefits attributed to *N. sativa* arise from both its raw and processed components. Among these, Thymoquinone (TQ) stands out as the principal bioactive compound. This potent substance is abundantly present in the oil and extracts derived from *N. sativa*, contributing significantly to its therapeutic efficacy (Asal Ulus et al 2018). *N. sativa* is widely recognized for its capability to boost the functionality of critical antioxidant enzymes. Among these enzymes are glutathione peroxidase (GPx), glutathione-S-transferase, and catalase, all of which play essential roles in maintaining cellular health and protecting against oxidative damage. This improvement in enzyme functionality is crucial in mitigating the harmful effects of toxins ingested through the diet and in curbing the formation of reactive oxygen species (ROS), which contribute significantly to oxidative stress (Karimi et al 2019). These antioxidant properties underscore the plant's potential in protecting cellular structures from oxidative damage. Additionally, the potent anti-inflammatory properties of black cumin, particularly attributed to its main constituent, thymoquinone (TQ), are among its most significant pharmacological traits. These attributes collectively contribute to its therapeutic potential in managing oxidative stress and inflammation (Dwita et al 2019).

Considering the advantageous neuroprotective, anti-inflammatory, and free radical-scavenging characteristics of *N. sativa*, this research was designed to assess its protective capabilities against the toxicity induced by acrylamide in rats. The study sought to explore how *N. sativa* could mitigate the harmful effects of ACR exposure, focusing on its potential to shield neural tissue, reduce inflammation, and neutralize free radicals.

Material and Methods

In this study, 32 healthy 8-week-old male Wistar Albino rats of comparable body weights were utilized. The subjects acquired from the Selcuk University Experimental Medicine Research and Application Center were housed with eight rats in each cage during the study. Prior to initiating the experiment, the overall health condition of the subjects was assessed. Next, the researchers sorted the rats into four distinct groups, ensuring that each group had similar average weights. Throughout the 15-day experimental period, the subjects were kept in optimal living conditions. The environmental conditions for the experiment were meticulously maintained, featuring a regulated temperature of 23±2°C, a humidity level maintained at 50±10%, and a 12-hour light/dark cycle. The rats were kept in plastic cages and had unrestricted access to standard rat chow and fresh water, which were replenished daily. This research adhered to ethical standards and received approval from the Selcuk University Experimental Medicine Research and Application Animal Ethics Committee, with the approval number 2017-11, granted on March 21, 2017.

In this study, the subjects were organized into four evenly sized groups: the control group (C), the acrylamide group (A), the *N. sativa* group (NS), and the group receiving both acrylamide and *N. sativa* (ANS). During the 15-day experimental period, 40 mg/kg acrylamide (Sigma) was given to the A and ANS groups via gavage (Yener et al 2013, Erdemli et al. 2019, Tabeshpour et al 2019). Additionally, *N. sativa* oil (Botalife) was given to the NS and ANS groups at a dose of 10 mg/kg per day orally via gavage throughout the study (Mehri et al 2014, Karimi et al 2019).

Following the experiment, the subjects underwent general anesthesia (thiopental anesthesia 40 mg/kg) for blood sample collection through cardiac puncture. The samples were then collected in both anticoagulant (EDTA) containing tubes and non-anticoagulant tubes. The blood samples were centrifuged (4000 rpm for 10 min)(Hettich Universal 32R), and their serums were separated. The samples were preserved at -80°C prior to analysis to measure levels of TNF-α, IL-6, IL-1, IL-10, SOD, GSH, and MDA. The lives of the animals whose blood was drawn were terminated under anesthesia by cervical dislocation.

In this study, the levels of IL-6, IL-1, IL-10, TNF-α, MDA, GSH, and SOD in the collected serum samples were measured. This was accomplished using commercially available kits from BT Lab, and the measurements were conducted with an ELISA device (ELx800 Bio-Tek Instruments, Winooski, VT, USA). These procedures ensured precise and reliable quantification of the biomarkers in question.

Statistical Analysis

The statistical analyses of the results obtained at the end of the study and the determination of the significance of differences between groups were performed using the SPSS 22.0 software package. The comparison of data obtained from the groups was carried out using a one-way ANOVA test. All values were presented in the table as mean ± SD. After assessing the homogeneity of variances, Duncan's Multiple Range test was used to test the significance between groups when the P-value was below 0.05 in the variance analysis.

Results

The details regarding the levels of serum cytokines, including IL-1, IL-6, IL-10, and TNF-α, along with the concentrations

Table 1.Effect of oral N. sativa administration on MDA, GSH and SOD levels in rats with 15-day experimental acrylamide toxicity (X ± SEM, n = 8).

Groups	MDA (nmol/ml)	GSH (mg/l)	SOD (ng/ml)
C	0.78 ± 0.28^{c}	5.30 ± 0.51^{ab}	4.95 ± 0.74^{a}
NS	0.85 ± 0.10^{c}	5.87 ± 0.65^{a}	5.23 ± 0.84^{a}
A	1.65 ± 0.14^{a}	2.69 ± 0.57^{c}	1.28 ± 0.23^{b}
ANS	1.19 ± 0.11^{ab}	3.84 ± 0.37^{bc}	3.31± 0.87^{ab}
p	0.000	0.002	0.004

^{a,b,c} Statistical analysis reveals a significant distinction ($P < 0.05$) between the mean values indicated by different letters within the same column for the identical parameter, emphasizing the variability and statistical significance of the observed differences. A = Acrylamide group; ANS = Acrylamide, and N. sativa applied group; C = Control group; GSH = Glutathione; MDA = Malondialdehyde; NS = N. sativa group; SOD = Superoxide dismutase.

Table 2. Effect of N. sativa administration on TNF-α, IL-6, IL-1 and IL-10 in rats with 15-day experimental acrylamide toxicity (X ± SEM, n = 8).

Groups	TNF-α (pg/ml)	IL-6 (pg/ml)	I IL-1 (pg/ml)	IL-10 (pg/ml)
C	78.79 ± 9.39^{b}	18.26 ± 0.38^{b}	23.85 ± 0.05^{c}	97.18 ± 7.13^{a}
NS	75.82 ± 7.86^{b}	19.26 ± 2.18^{b}	27.37 ± 1.09^{c}	94.66 ± 6.64^{a}
A	114.99 ± 10.31^{a}	41.05 ± 6.84^{a}	45.17 ± 0.43^{a}	46.29 ± 3.64^{b}
ANS	92.69 ± 8.14^{ab}	30.73 ± 2.86^{a}	32.97 ± 0.3^{b}	77.84 ± 5.80^{ab}
p	0.023	0.001	0.002	0.000

^{a,b,c} Statistical analysis reveals a significant distinction ($P < 0.05$) between the mean values indicated by different letters within the same column for the identical parameter, emphasizing the variability and statistical significance of the observed differences. A = Acrylamide group; ANS = Acrylamide, and N. sativa applied group; C = Control group; GSH = Glutathione; MDA = Malondialdehyde; NS = N. sativa group; SOD = Superoxide dismutase.

of MDA, a key indicator of lipid peroxidation, and the antioxidants GSH and SOD are presented in Tables 1 and 2. These tables offer a thorough summary of the biochemical parameters evaluated in this research.

In the study, serum MDA levels in rats exposed to acrylamide (group A) showed a marked increase compared with the remaining three groups ($P < 0.05$). Although the MDA level in the ANS group, which was administered *N. sativa* for protective purposes against acrylamide toxicity, was higher than in the C and NS groups without statistical significance, it was significantly lower relative to the A group ($P< 0.05$). GSH and SOD levels of antioxidant enzymes were observed to be statistically substantially reduced ($P< 0.05$) in group A relative to the values in the other three groups (except for ANS for GSH). The *N. sativa* treated ANS group exhibited notably higher SOD levels than those observed in group A ($P < 0.05$).

The study determined that acrylamide statistically significantly increased TNF-α, IL-6, and IL-1 levels in sera in group A compared to those in the C and NS groups ($P < 0.05$). Nonetheless, the application of *N. sativa* in conjunction with acrylamide led to a numerical reduction in TNF-α and IL-6 levels, and a notable decrease in IL-1 levels within the ANS group when contrasted with the A group, showing statistical significance ($P < 0.05$). The level of IL-10, known to be an anti-inflammatory cytokine, was found to be lower in group A than in all other groups ($P < 0.05$).

Discussion

Many studies show that acrylamide, like many toxic substances, causes lipid peroxidation in the cell (Yousef and El-Demerdash 2006, Allam et al 2011, Hamdy et al 2012, Zhang et al 2013, İnce et al 2018). Research on acrylamide toxicity has revealed that acrylamide exposure increases free oxygen radicals, resulting in oxidative stress because of the disturbance of the oxidant/antioxidant balance (Pan et al 2015). Additionally, various studies have discovered that acrylamide exposure decreases antioxidant enzyme levels such as SOD, catalase, and GSH in various tissues by inducing lipid and protein oxidation (Zamani et al 2018, Tabeshpour et al 2020, Amirshahrokhi 2021).

ACR, being highly soluble in water, rapidly disperses throughout the digestive tract after ingestion. It conjugates with GST/GSH to form an acrylamide-glutathione complex, thus reducing its adverse effects. However, the effectiveness of this conjugation in the digestive tract is directly related to the GST and GSH levels in the tissue (Van Lieshout et al 1998).

This study observed that orally administered acrylamide caused significant oxidative stress (Table 1). Our results are consistent with existing studies on this subject (Alturfan et al 2012, Zhang et al 2013, Zargar et al 2016, Uthra et al 2017, Acaröz et al 2018, Pan et al 2018). The elevated MDA level indicates a possible decline in the endogenous antioxidant capacity. The significant rise in MDA level and the decline in GSH and SOD levels observed in group A can be attributed to the pronounced impact of ACR-induced oxidative stress. Previous research has shown that when acrylamide accumulates in the body, it binds to glutathione (GSH), a molecule essential for combating oxidative stress. This binding leads to an elevated production of reactive oxygen species (ROS), which in turn inflicts damage on cellular macromolecules (Pradeep et al 2007, Uthra et al 2017). Some scientists argue that the increase in MDA level due to acrylamide exposure results from a decrease or depletion of glutathione at critical levels (Zargar et al 2016, Uthra et al 2017, Şengül et al 2021). The results gathered from this research further corroborate this perspective.

The decrease in GSH level is a prominent finding observed in animals subjected to acrylamide toxicity. The depletion of GSH is thought to be due to the higher consumption rate in reactions with hydrogen peroxide in phase II reactions catalyzed by glutathione S-transferase and conjugation with acrylamide and glycoside (Paulsson et al 2005, Kopańska et al 2015). Additionally, Zhu et al (2008) reported a decrease

in SOD activity in the nervous system of 10-week-old rats after acrylamide administration every three days. This effect is probably due to the oxidation of SOD by the overproduced superoxide ion. All these suggest that acrylamide induces greater activity in the antioxidant system and that elevated doses of acrylamide administered for extended periods increase oxidative stress symptoms (Semla et al 2017).

Studies on thymoquinone, the primary compound present in *N. sativa*, have highlighted the antioxidant properties of this plant (Hosseinzadeh et al 2007, Kanter et al 2006). When thymoquinone is converted into its reduced form, thymohydroquinone, it functions as a powerful electron donor. This conversion enables it to neutralize both hydroxyl radicals (OH-1) and superoxide radicals effectively. As a result, this process impacts the polyunsaturated fatty acids within the cell membrane, which is crucial for preserving cellular stability and integrity. This mechanism highlights thymoquinone's remarkable ability to combat free radicals and protect cells from oxidative damage (Staniek et al 2010, Khither et al 2018).

Utilization of *N. sativa* oil in this research to lessen or counteract the harmful impacts of ACR highlights its significant role as a potent natural source of antioxidants. When the data obtained in the study were examined, it was found that *N. sativa* lowered the MDA level, an indicator of lipid peroxidation, while increasing antioxidant enzymes like GSH and SOD in the ANS group (Table 1). The obtained data reveal the antioxidant activity of *N. sativa* and support existing studies on this subject. In Mehri et al (2015) study, it was discovered that TQ treatment significantly reduced the elevated MDA concentration in the cerebral cortex of rats administered ACR (50 mg/kg for 11 days). Another study found that administering varying doses of TQ to rats suffering from acrylamide toxicity significantly reduced MDA concentrations and elevated GSH levels in the treated group.

Inflammation and immune response are very active in forming the early response to tissue damage. The liver's capacity to produce and remove cytokines is crucial for their cellular metabolism. Therefore, determining cytokine concentrations in blood serum is considered a biomarker in toxicity studies (Lacour et al 2005). TNF-α, an inflammatory cytokine, promotes the development of inflammation by stimulating ROS generation within the cell via the transcription of mitogen-activated protein kinase (MAPK) (Zhou et al 2009, Jeon et al 2013). Elevated ROS levels in the cell can activate p38, which plays a role in apoptosis and phosphorylation, thereby triggering the inflammatory cascade (Bao et al 2010, Bai et al 2013). Oxidative stress in the organism can enhance NF-kB activation and the transcription of the TNF-α gene (Chandel et al 2000, Daniele et al 2015).

Consistent with findings from research on ACR toxicity, it is proposed that elevated ROS production, which varies with the dose and length of ACR exposure, is directly associated with heightened inflammatory parameters (Zhao et al 2017, Pan et al 2018). Studies have reported elevated concentrations of inflammatory cytokines IL-1, IL-6, and TNF-α in blood serum and/or plasma following ACR exposure (Abdel-Daim et al 2015, Alturfan et al 2012, Zhang et al 2013). ACR has been shown to damage intestinal tissue in rats through upregulation of IL-1, TNF-α, and IL-2 expression (Yang et al 2019, Amirshahrokhi 2021).

Consistent with previous studies, the findings from this research indicate that exposure to ACR results in a substantial elevation in serum levels of inflammatory cytokines. Specifically, there is a notable increase in the concentrations of TNF-α, IL-1, and IL-6. These changes underscore the significant inflammatory response initiated by ACR exposure. These results underscore the significant inflammatory response provoked by acrylamide exposure. These findings suggest that ACR triggers an inflammatory response within the cell. This study found elevated concentrations of cytokines like TNF-α, IL-1, and IL-6 in the ACR-applied group and a decrease in the level of IL-10, an anti-inflammatory cytokine. Simultaneous treatment with *N. sativa* with ACR was observed to improve these parameters due to its anti-inflammatory activity (Table 2). IL-10 plays a crucial role in reducing tissue inflammation by either inhibiting the production of several inflammatory cytokines and chemokines or by curbing the activity of TNF-α. Experimental research has demonstrated that IL-10 can lessen the severity of inflammation caused by toxic substances, primarily by decreasing the levels of various inflammatory markers (Zhang et al 2013, Yang et al 2019, Amirshahrokhi 2021, Sayed et al 2022). This effect of *N. sativa* suggests it may suppress acute or chronic inflammation in the organism.

Conclusion

The data obtained at the end of the trial show that the application of *N. sativa* does not cause any adverse effects on the organism when used alone. It was found to have a remarkable curative effect on the parameters examined in the intoxicated group. Based on the study's findings, N. sativa, commonly used as a spice for its potent antioxidant and anti-inflammatory properties, is believed to mitigate or diminish the detrimental impact of ACR. It seemed that *N sativa* significiantly improved the increase of MDA levels altered by ACR and increase antioxidant levels, and partially attenuate the changes in L-1 levels induced by ACR. Its capacity to mitigate inflammation induced by ACR and mitigate oxidative stress underscores its potential therapeutic applications.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Author Contributions

Motivation/Concept: MO, TK; Design: MO, TK; Control/Supervision: MO, TK; Data Collection and Processing: MO, TK Analysis and Interpretation: MO, TK; Literature Review: MO, TK; Writing the Article: MO, TK; Critical Review: MO, TK

Ethical Approval

The Ethical Committee of Selçuk University, Faculty of Veterinary Medicine, Türkiye (No 2024/062) approved this study.