

PROTECTIVE EFFECT OF *Nigella sativa* ON SOME HEMATOLOGICAL PARAMETERS IN RATS EXPOSED TO ACRYLAMIDE

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ABSTRACT

Acrylamide (ACR), a well-known toxic substance, is found in heated foods and processed foods at high temperatures. ACR is a neurotoxic and carcinogenic agent that poses a greater risk to human and animal health. The aim of this study was to determine the effect of *Nigella sativa* (NS) oil on some hematological and immunological parameters against ACR-induced toxicity in rats. Male Wistar albino rats (N=32) with an average weight of 358±8.10 grams were divided into four equal groups: control (C), Acrylamide (ACR, 40 mg/kg, gavage), *Nigella Sativa* (NS oil 10 ml/kg, gavage), and Acrylamide + *Nigella Sativa* (ACR+NS oil). In the blood samples taken from animals, red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin amount, hematocrit value (Htc), platelet (Plt) count, leukocyte percentages and mean red blood cell volume (MCV), mean red blood cell hemoglobin (MCH) and mean red blood hemoglobin were determined together with the concentration of MCHC hemoglobin, alpha-naphthyl acetate esterase (ANAE) positive peripheral blood lymphocyte ratios. A significant decrease in RBC, PLT, HB and HTC levels and lymphocyte percentage, and an increase in WBC count and neutrophil percentage were detected in rats in the ACR group. NS oil applied simultaneously with ACR tended to improve these parameters. These results confirm the effects of ACR on some hematological and immunological parameters and reveal that NS oil has a protective effect against acrylamide-induced toxicity in rats.

Keywords: Acrylamide, ANAE, haemogram, immune system, N. Sativa.

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INTRODUCTION

Various industries, including packaging, mining, paper production, paint production, water treatment, textiles, and cosmetics, use Acrylamide (ACR), an artificial monomer. Researchers widely recognize ACR as a neurotoxic, carcinogenic, and genotoxic compound (Acaroz *et al.* 2018; Guo *et al.* 2020). We acknowledge that consuming high-carbohydrate foods cooked at high temperatures significantly exposes us to ACR, as these foods contain ACR (Mehri *et al.* 2014). The Maillard reaction produces ACR when the carbonyl group of reduced sugars reacts with the amino group of free asparagine in foods heated for an extended period above 120 °C (Can *et al.* 2016; Kavuşan and Serdaroglu, 2019).

Animals and humans' hematopoietic systems are highly susceptible to the harmful effects of environmental toxins and drugs. After ACR is taken into the organism, it is absorbed through the intestinal tract and directed towards the circulation as well as various target organs. It causes various toxic effects by interacting with DNA,

neurons, basic enzymes, and hemoglobin (Ghorbel *et al.*, 2015). Exposure through oral ingestion, inhalation, or skin contact results in higher concentrations of ACR in the bloodstream compared to other tissues. According to Ghorbel *et al.* (2015), the presence of ACR and glycidamide hemoglobin adducts in the blood is a reliable indicator of ACR-induced toxicity. Reactive oxygen species (ROS) can cause oxidative damage to erythrocytes due to the high concentration of polyunsaturated fatty acids in their membranes and the redox-active hemoglobin (Hb) molecules, which serve as a significant source of ROS (Shrivastava *et al.* 2019). Experimental studies have reported that the intake of ACR leads to oxidative stress, has harmful effects on the nervous system and genetic material, and can potentially cause cancer in both animals and humans (Can *et al.* 2016; Shrivastava *et al.* 2019). The body readily absorbs and undergoes metabolism of ACR, which then enters the bloodstream. It can interact with hemoglobin, plasma proteins, and certain enzymes, while also causing DNA damage as it spreads to different organs (Baum *et al.*

2008; Abdel-Daim *et al.* 2020). Researchers have conducted extensive research on the use of protective agents, such as natural antioxidants, to counteract the toxic effects of ACR. *Nigella sativa* (NS) is extensively employed globally as a preservative and food additive, as well as for combating diverse ailments (Karimi *et al.* 2019). The oil and seed of NS have extensive applications, particularly in oriental medicine, due to their various beneficial properties, such as diuretic, antihypertensive, antidiabetic, anticancer, immunomodulatory, analgesic, antimicrobial, anthelmintic, anti-inflammatory, spasmolytic-bronchodilator, gastroprotective, hepatoprotective, renal protective, antioxidant protective, and therapeutic effects (Ahmad *et al.* 2013). Thymoquinone (TQ) is the primary chemical molecule in NS that exhibits the highest pharmacological activity. Neuroprotective substances lessen the harmful effects of food toxins by controlling the activity of antioxidant enzymes like glutathione peroxidase (GPx) and glutathione-S-transferase catalase and acting as ROS scavengers in different body parts (Mehri *et al.* 2014).

Despite reports of acrylamide's potential toxicity, there have been few studies examining changes in blood composition. This study aimed to investigate the impact of NS, known for its potent antioxidative and anti-inflammatory properties, on certain hematological parameters and the ratios of ANAE-positive lymphocytes in experimental rats exposed to ACR.

MATERIALS AND METHODS

Animals: The Selcuk University Experimental Application and Research Center conducted animal experiments. The Ethics Committee of the Selcuk University Veterinary Faculty Experimental Animal Production and Research Center approved the study protocol with Approval No. 2017/11. The experimental procedures were conducted in compliance with the animal welfare regulations of the European Economic Community, specifically the directives 86/609/CEE and 2010/63/EU. A total of 32 healthy male Wistar Albino rats, all with similar body weights (358 ± 8.10 gr), were utilized in the research. The experimental animal unit specifically housed the rats in plastic rat cages. The room temperature was maintained at 23 ± 2 °C, with a relative humidity of $50 \pm 10\%$. The rats were exposed to a 12-hour night/daylight cycle and had unrestricted access to standard rat chow. The rats were given a daily supply of freshwater, approximately 50 ml per day per rat, which was available to them at all times.

Experimental design: A total of 32 healthy male Wistar Albino rats were allocated into four equal groups: control (C), Acrylamide (ACR), *Nigella Sativa* (NS oil), and Acrylamide + *Nigella Sativa* (ACR+NS oil). Hatipoglu *et*

al. in 2024 described the administration of 40 mg/kg of acrylamide (Sigma) via gavage to the ACR and ACR+NS groups throughout the 15-day trial. The NS and ACR+NS groups were administered 10 ml/kg of *N. sativa* oil (Botalife) via gavage throughout the trial, as reported by Karimi *et al.* in 2019. For a period of 15 days, we administered NS at a dosage equivalent to 1.25 percent of the daily food ration. ACR was prepared daily before administration, specifically during the trial period. The proficient researcher utilized gastric feeding needles of the ball type to administer ACR and NS to all animals via gavage, ensuring equal and complete dosages. The researcher carefully inserted the feeding needles, attached to syringes, into the lower esophagus through the rats' mouths. We used these needles to avoid entering the trachea and to reduce harm to the oral cavity and esophagus.

Hematological parameters and differential leukocyte counts: At the end of the study, we collected blood samples from the subjects in the different groups while they were under general anesthesia, specifically thiopental anesthesia at a dosage of 40 mg/kg. The blood was obtained by puncturing the heart and collecting it in tubes containing an appropriate anticoagulant (EDTA). The blood samples were analyzed to determine the levels of leukocytes (WBC), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte counts. The hematological analysis results were acquired utilizing an automated blood count device, specifically the Mindray BC800. Furthermore, the smears made from the blood samples were used to determine the ratios of ANAE-positive lymphocytes.

Alpha-naphthyl acetate esterase (ANAE) demonstration: Two smears were made from the drawn blood samples specifically for this purpose. Smears that had been air-dried were fixed for three minutes at -10°C in a glutaraldehyde-acetone fixing solution ($\text{pH} = 4.8$). After dissolving 20 mg of the substrate (alpha-naphthyl acetate, N-8505-Sigma) in 0.8 ml of acetone (Merck), the mixture was gradually added to 80 ml of $\text{pH} 5.0$ buffered phosphate solution. At room temperature, the smears were thereafter allowed to dry. The buffered phosphate solution containing the substrate was then mixed with 2.4 ml of 4% sodium nitrite (S-3421, Merck) solution and 4.8 ml of the hexazotized pararosaniline mixture, which was made by preserving 2.4 ml of pararosaniline (P-3750, Merck) (1 g of pararosaniline, 20 ml of distilled water, and 5 ml of concentrated HCl) solution for two minutes. The prepared solution was filtered and maintained under strict supervision for two hours in the incubation solution after its pH was adjusted to 5.8 using 1N NaOH solution. The incubation period was stopped at the emergence of

reddish-brown granules (Figure 1, arrow), and methyl green core staining was applied to the preparates after three distilled water washes. Using an x100 objective (Leica DM2500, Switzerland), 200 lymphocytes from each sample were counted to determine the ANAE+ lymphocyte ratios (Figure 1) (Kisadere *et al.* 2022). At the conclusion of the study, statistical analysis of the data and analysis of variance were performed to determine the significance of differences between groups using the SPSS 22.0 package program ($P \leq 0.05$).

RESULTS

At the end of the experiment, some hematological parameters and differential leukocyte counts determined in the research groups in the blood samples taken are given in the tables (Figure 1, Table 1).

When looking at the hematological parameters shown in Figure 1, the RBC, HGB, HCT, PLT, and MCV values in the rats in the ACR group were significantly lower than those in the control group ($P \leq 0.05$). In addition, there was a notable rise in white blood cell (WBC) levels in the ACR group ($P \leq 0.05$). There were a few more red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), and mean corpuscular volume (MCV) in the ACR+NS group of rats than in the ACR group. However, this increase was not considered statistically significant ($P > 0.05$) when compared to the control group. Nevertheless, the PLT value in the ACR+NS group exhibited a statistically significant rise in comparison to the ACR group ($P \leq 0.05$) and maintained comparable levels when compared to the control group ($P > 0.05$). The white blood cell (WBC) count in the ACR+NS group of rats showed a tendency to decrease compared to the ACR group ($P \leq 0.05$).

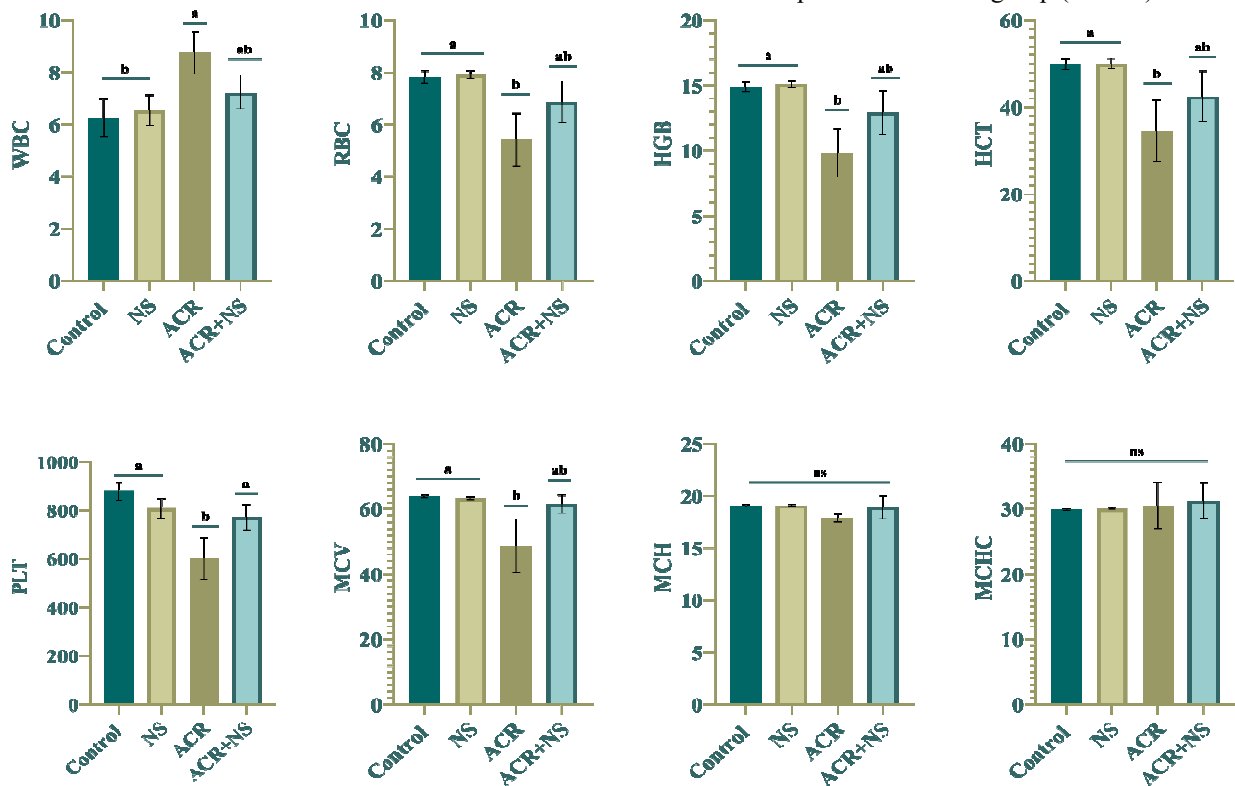


Figure 1. Some hematological parameters in experimental groups, $X \pm SX$. a, b; $P \leq 0,05$

Table 1. Differential leucocyte counts (%) and ANAE positive lymphocyte counts (%) in experimental groups, $X \pm SX$

Parameters (%)	C	NS	ACR	ACR+NS
Lymphocyte	63.91 \pm 4.56 ^a	61.75 \pm 1.45 ^a	32.21 \pm 5.72 ^b	41.95 \pm 4.16 ^b
Monocyte	2.34 \pm 1.06	3.36 \pm 0.94	2.77 \pm 0.40	3.70 \pm 0.63
Neutrophile	27.45 \pm 3.69 ^b	31.43 \pm 3.50 ^b	60.16 \pm 6.05 ^a	49.71 \pm 4.15 ^a
Eosinophile	3.81 \pm 0.71	3.83 \pm 1.01	2.38 \pm 0.46	1.97 \pm 0.41
Basophile	2.45 \pm 0.63	1.81 \pm 0.48	2.28 \pm 0.84	3.08 \pm 1.24
ANAE	59.33 \pm 2.23 ^{bc}	61.67 \pm 2.36 ^{ab}	68.17 \pm 2.06 ^a	62.50 \pm 2.22 ^{ab}

a, b, c; $P \leq 0,05$

Table 1 shows the leukocyte percentages. The ACR group had a significantly lower lymphocyte percentage compared to the control group ($P \leq 0.05$). The neutrophil percentage in the same group exhibited a statistically significant increase compared to the control group ($P \leq 0.05$). In contrast, the ACR+NS group showed a slight improvement in lymphocyte and neutrophil levels compared to the ACR group. However, these differences did not reach statistical significance ($P > 0.05$). The ACR

group showed a significant increase in the ratio of ANAE-positive lymphocytes compared to the control group ($P \leq 0.05$). Within the ACR+NS group, there was a noticeable trend of reduced ANAE-positive lymphocyte count compared to the ACR group, but an increase compared to the control group. Nevertheless, neither of these alterations exhibited statistical significance ($P > 0.05$).

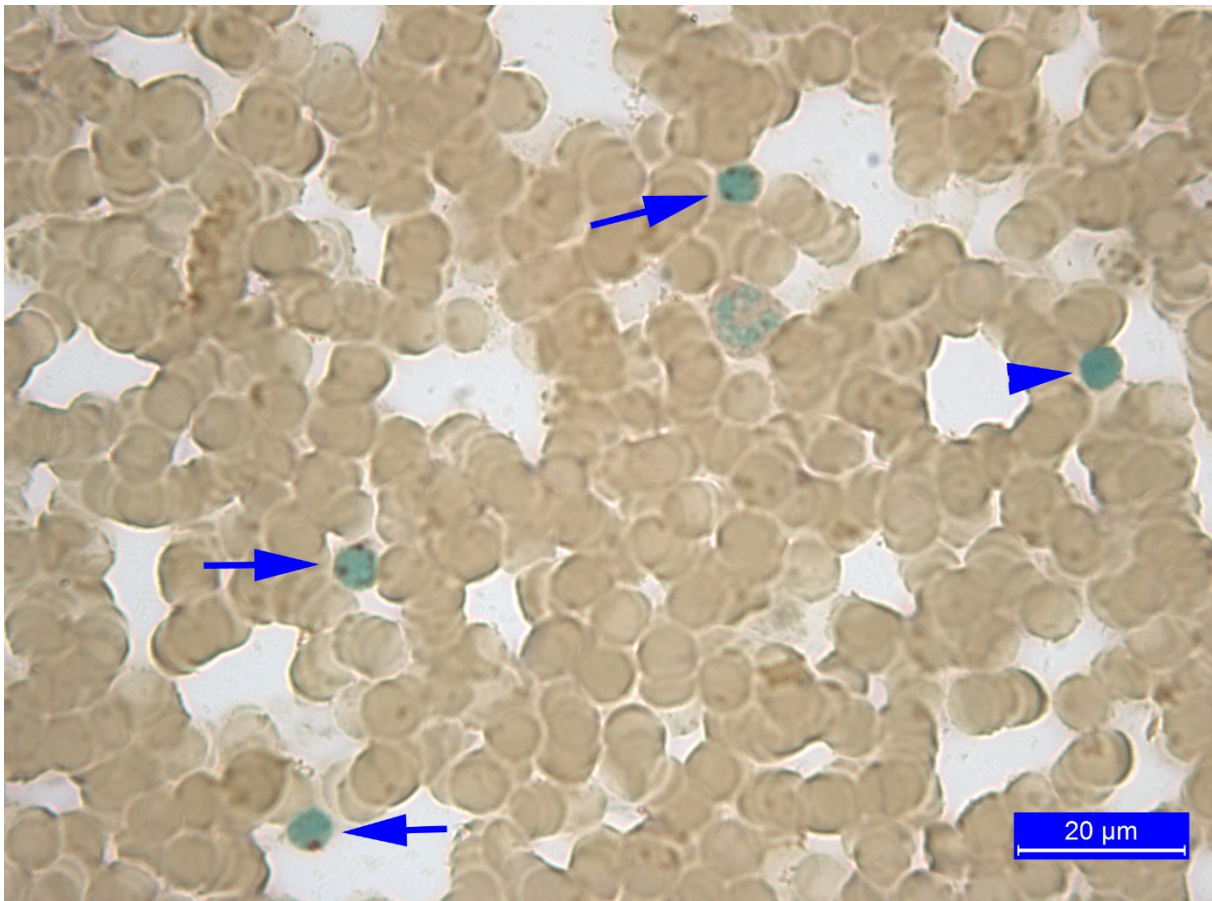


Figure 2. ANAE positive and negative lymphocyte. Arrows: indicates ANAE positive lymphocytes, Arrowhead: indicates ANAE negative lymphocytes.

DISCUSSION

It is still not known exactly what kind of damage ACR, which is taken into the organism in various ways, may cause the organism. Therefore, especially food-borne ACR is an important problem (Morales *et al.* 2014; Arusoğlu 2015). The mechanism of acrylamide toxicity begins when there is an imbalance in the biological oxidant-antioxidant ratio and it results in the potential for oxidative stress to occur. In recent years, it has been shown that ACR induces oxidative stress (Nowak *et al.* 2020). ACR is not only neurotoxic, it can also alter hematological parameters by damaging erythrocyte

membranes, producing micronuclear erythrocytes, and also altering blood viscosity parameters (Arihan *et al.* 2011).

In this study, it has been observed that the erythrocyte count, hemoglobin amount, hematocrit value, MCV and platelet count decreased significantly in rats with ACR toxicity compared to the other three groups (C, NS, and ACR+NS). The decrease in erythrocyte count, hemoglobin amount, and hematocrit value observed in the ACR group indicate anemia. It is well known that cytokines released by inflammatory cells induce anemia and result in reduced iron consumption. Iron deficiency is the main cause of microcytic hypochromic anemia (Raju *et al.* 2015, Mahmood *et al.* 2016.). Microcytic

hypochromic anemia is an indicator of a decreased response to erythropoietin. Research on this subject reveals that this may be an effective mechanism in chronic renal failure that may occur only as a result of AA administration (Mahmood *et al.* 2015). However, inflammation that develops due to toxicity in the organism causes a change in the recycling dynamics of iron. Here, iron does not promote the formation of red blood cells in the bone marrow; rather, it is stored in the reticuloendothelial system (Mahmood *et al.* 2016). Benziane *et al.* (2019) state that the number of RBCs in the treated groups after ACR application was lower than in the control group, and this observed decrease reveals anemia.

Previous research has shown that ACR can alter certain hematological parameters (Rawi *et al.* 2012; Ghorbel *et al.* 2015; Raju *et al.* 2015; Wu *et al.* 2021). An investigation into this topic found that ACR treatment reduced hemoglobin levels, red blood cell count, and lymphocyte count in mice (Rawi *et al.* 2012). Consistent with previous research, this study's findings show that ACR has a significant impact on hematological parameters, resulting in their reduction. Şekeroğlu *et al.* (2017) found that ACR causes a significant decrease in all hematological parameters, except the mean corpuscular hemoglobin concentration. The study found that applying argan oil to the acrylamide-treated group significantly increased the previously reduced hematological parameter levels caused by ACR. According to reports, using ACR reduces red blood cell membranes' ability to resist acid and initiates the process of lipid peroxidation (Tarskikh 2006). Farouk *et al.* (2021) investigated the toxicity of hemograms. They found a significant decrease in red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration, hematocrit percentage, and neutrophil and lymphocyte counts. The study used ACR (Farouk *et al.* 2021). The findings of Lebda *et al.* (2015) support the presence of low levels of red blood cells (RBC), hemoglobin (Hb), and hematocrit. The researchers emphasize that the ACR binding to Hb causes changes in hemogram parameters by depleting a portion of the Hb molecules. This process produces adducts that reduce the amount of hemoglobin in the bloodstream, potentially contributing to anemic conditions. ACR can lower the amount of Hb in the blood by chemically attaching to cysteine residues and combining with Hb's sulfhydryl group to form compounds (Barber *et al.* 2001). Furthermore, the reticuloendothelial system may sequester iron instead of releasing it to stimulate red blood cell production in the bone marrow. This is due to ACR-induced inflammation, which alters iron recycling (Mahmoud *et al.* 2016).

It has also been reported that after ACR application, changes accompanied by a decrease in the physicochemical properties of biological membranes are

observed (Tarskikh 2006). Rivadeneyra-Dominguez *et al.* (2018) showed that erythrocyte, hemoglobin, hematocrit, MCV, and leukocyte levels decrease, supporting previous reports (Ghorbel *et al.* 2015; Benziane *et al.* 2019) showing similar microcytic anemia in rats treated with 25 and 50 mg/kg acrylamide. They report that platelets were reduced only in rats treated with 50 mg/kg. In their study, Mahmood *et al.* (2016) reported that most of the hematological parameters in the groups treated with ACR did not show a significant change compared to the control group, but they reported that the levels of MCH, MCV, platelet, and HCT were significantly higher than the control group. Imai and Kitahashi (2014) observed a decrease in RBC and HCT, which caused experimental acrylamide toxicity. In a previous study, it has been argued that the hematological changes (reduction in RBC and Hb and increased MCV) reported in rats and mice are associated with bone marrow toxicity of acrylamide (Paulsson *et al.* 2005).

White blood cells (WBC), also called leukocytes, are the most important elements of the immune system. In the study of Mahmood *et al.* (2016), in which they investigated ACR toxication, they determined that the number of leukocytes in the intoxicated group was significantly higher than in the untreated rats ($p \leq 0.05$). Mahmood *et al.* (2016) found in their study that the total number of WBCs was significantly higher in the ACR group than in the control group and showed the activation of the immune system, which can also be considered as the incidence of inflammation. Similarly, Ghorbel *et al.* (2015) also reported statistically insignificant increases in the number of WBCs. According to results from another trial, the group receiving ACR showed higher WBC counts and lower RBC, HB, and blood platelet (PLT) counts in comparison to the control group (Raju *et al.* 2015). The increase in the number of WBCs we determined in this study is consistent with the above data. Researchers interpret this as indicating that the immune system is activated by the ACR and indicates the presence of inflammation. On the other hand, there are also studies that reported that the decrease determined in total WBC may be due to the rapid destruction and decreased production of leukocytes related to the immunosuppressive effect of ACR (Lebda *et al.* 2015). It has been reported that WBC, lymphocytes, and monocytes decreased in female BALB/c mice administered 36 mg/kg ACR for 30 days (Fang *et al.* 2014). Bousalham *et al.* (2015) reported that although there is an increase in the number of eosinophils, there is no significant change in other hematological parameters (WBC, monocytes, basophils, and platelets).

In this study, it was determined that NS oil applied to animals exposed to ACR experimentally reduced the decrease in some hematological parameters (erythrocyte count, hemoglobin amount, hematocrit

value, MCV and platelet count) compared to the experimental group (ACR), which administered toxication. The positive effect of NS on the parameters of the animals treated with ACR should be due to its effective antioxidant properties. This is because NS is considered a powerful systemic radical scavenger. The beneficial effect of NS on hematological parameters may be because it blocks oxidative damages in DNA and reduces glycation of membrane proteins reduces lipid peroxidation and prevents free radical formation related to inhibition of some enzymes (Karimi *et al.* 2019).

ACR at a level similar to LD50 may play important roles in controlling immunological responses and encouraging the maturation of naïve T cells into Th2 lymphocytes during the early immune response, according to Descamps-Latscha and Witko-Sarsat (1999). In their study, Benzianea *et al.* (2019) observed an increase in the population of monocytes and macrophages following the application of ACR at a concentration of 10 mg/L or LD50. These immune cells regulated their immune responses by releasing substantial quantities of cytokines (Benziane *et al.* 2019). Zaidi *et al.* (1994) conducted a study using a rat model to investigate the immunotoxic effects of ACR. They found that ACR causes a reduction in the number of lymphocytes in the bloodstream. This decrease in lymphocytes may be attributed to the suppression of both humoral and cell-mediated immunity by ACR. Benzianea *et al.* (2019) observed notable elevations in leukocyte and lymphocyte levels, which were found to be dose-dependent in relation to ACR toxicity. In their study, Benzianea *et al.* (2019) observed a rise in Kupffer cells and an augmentation in pigment accumulation. Kupffer cells are a type of macrophage, and the liver serves as the primary reservoir for macrophages in the body. According to their findings, the count of eosinophilic polynuclear cells, which are cells that promote inflammation, decreased. In the initial immune response (which is boosted by the administration of ACR at a concentration of 10 mg/L or LD50), these cells are thought to be essential for regulating immune responses and supporting the maturation of naive T cells into Th2 lymphocytes, as well as into B lymphocytes in subsequent immune responses. Monocytes and macrophages, which experience an increase following the implementation of ACR, secrete substantial quantities of cytokines, thus playing a crucial role in the regulation of immune responses (Benziane *et al.* 2019).

According to Gul *et al.* (2007), studying and assessing lymphocyte enzymes can offer valuable insights into the operational condition and development of the immune system. This information can be beneficial for diagnosing immune system disorders. ANAE, an enzyme found in lysosomes, is believed to be accountable for the cytotoxic effects of T-lymphocytes and the phagocytic activity of monocytes (Donmez *et al.* 2019). This enzyme has been observed in fully developed,

immune-competent T-lymphocytes of various species (Basso *et al.* 1980; Dönmez Hasan Hüseyin *et al.* 2007; Dönmez H. H. and Sur 2008; Dönmez N *et al.* 2016; Kisadere *et al.* 2022). Prior research has collected data regarding the immediate harmful effects on the immune system caused by large amounts of acrylamide. Several studies have reported notable decreases in the weight of lymphoid organs, particularly the mesenteric lymph nodes, spleen, and thymus (Zaidi *et al.* 1994; Yener *et al.* 2013; Fang *et al.* 2014; Zamani Ehsan *et al.* 2017; Zamani E. *et al.* 2017). A study utilizing ACR revealed a significant dose-dependent decrease in the number of alpha-naphthyl acetate esterase (ANAE) positive lymphocytes in both ileal Peyer's plaques and peripheral blood of all animals treated with acrylamide (Yener *et al.* 2013). In their study, Fang *et al.* (2014) found that administering acrylamide at doses of 4, 12, and 36 mg/kg/day for 30 days resulted in a significant decrease in the weight of the spleen and thymus in mice. They were investigating the toxic effects of acrylamide and observed a reduction in hematopoietic stem cells in mice given the highest dose of acrylamide (36 mg). Additionally, they noted significant changes such as the narrowing of germinal centers in the lymph nodes and the disappearance of germinal centers in the spleen (Fang *et al.* 2014). According to the same study, the researchers contend that the use of ACR resulted in an increase in the number of T-lymphocytes (CD3+, CD19-), while causing a decrease in the numbers of NK cells (CD3-, CD49-) and B lymphocytes (CD3-, CD19+). Academics contend that acrylamide exerts a harmful impact on both the cellular and humoral immune response. The data collected in this study indicate a significant increase ($P \leq 0.05$) in ANAE positive lymphocytes following the application of ACR. Although the total number of lymphocytes decreased in rats treated with ACR, there was an increase in T lymphocytes relative to B lymphocytes. This indicates that the organism responds to ACR by enhancing its cytotoxic activity in peripheral blood, rather than boosting its humoral immunity. The decrease in B lymphocyte ratio may indicate the migration of these cells from the peripheral blood to the secondary lymphoid organs, where they increase their activities. According to Fang *et al.* (2014), the administration of high doses of ACR resulted in an increase in the level of serum immunoglobulin, despite a decrease in B lymphocytes in the peripheral blood.

The alteration in the leukogram can be ascribed to the immunotoxic impact of ACR, resulting in a reduction in immune blood cells (Zamani E. *et al.* 2017). Our study found that the group treated with ACR experienced a decline in all hematological parameters, while *N. Sativa* approached the values seen in the control group. These findings align with previous studies conducted by Eze *et al.* in 2019. The enhancement in both hemogram and leukogram can be ascribed to an

augmentation in the erythropoiesis process, along with the robust antioxidant and anti-inflammatory properties of *N.Sativa*, which could potentially induce a favorable immune response (Karimi *et al.* 2019).

Conclusion: This research validates the impact of ACR on certain hematological and immunological markers. However, more research is required to determine the specific component of the immune system that is most impacted and to investigate the activity of genes responsible for the observed variances in immunological response. Furthermore, the findings of this research demonstrate that *N. Sativa* oil has a safeguarding effect against the harmful effects generated by acrylamide in rats.

Availability of data: The authors verify that the data substantiating the conclusions of this research may be found in the journal itself and its additional resources.

REFERENCES

- Abdel-Daim, M. M., F. I. Abo El-Ela, F. K. Alshahrani, M. Bin-Jumah, M. Al-Zharani, B. Almutairi, M. S. Alyousif, S. Bungau, L. Aleya and S. Alkahtani (2020). Protective effects of thymoquinone against acrylamide-induced liver, kidney and brain oxidative damage in rats. *Environ. Sci. Pollut. Res.* 27(30): 37709-37717. <https://doi.org/10.1007/s11356-020-09516-3>
- Acaroz, U., S. Ince, D. Arslan-Acaroz, Z. Gurler, I. Kucukkurt, H. H. Demirel, H. O. Arslan, N. Varol and K. Zhu (2018). The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. *Food Chem. Toxicol.* 118:745-752. <https://doi.org/10.1016/j.fct.2018.06.029>
- Ahmad, A., A. Husain, M. Mujeeb, S. A. Khan, A. K. Najmi, N. A. Siddique, Z. A. Damanhour and F. Anwar (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pac. J. Trop. Biomed.* 3(5): 337-352. [https://doi.org/10.1016/S2221-1691\(13\)60075-1](https://doi.org/10.1016/S2221-1691(13)60075-1)
- Arihan, O., N. B. Serinç, E. I. Gürel and N. H. Dikmenoglu (2011). Effects of oral acrylamide intake on blood viscosity parameters in rats. *Clin. Hemorheol. Microcirc.* 47(1): 45-52. <https://doi.org/10.3233/CH-2010-1364>
- Arusoğlu, G. (2015). Akriamid oluşumu ve insan sağlığına etkileri. *Akademik Gıda.* 13(1): 61-71.
- Barber, D. S., J. R. Hunt, M. F. Ehrlich, E. J. Lehning and R. M. LoPachin (2001). Metabolism, Toxicokinetics and Hemoglobin Adduct Formation in Rats Following Subacute and Subchronic Acrylamide Dosing. *Neurotoxicology.* 22(3): 341-353. [https://doi.org/https://doi.org/10.1016/S0161-813X\(01\)00024-9](https://doi.org/https://doi.org/10.1016/S0161-813X(01)00024-9)
- Basso, G., M. G. Cocito, G. Semenzato, A. Pezzutto and L. Zanesco (1980). Cytochemical Study of Thymocytes and T Lymphocytes. *Br. J. Haematol.* 44(4): 577-582. <https://doi.org/10.1111/j.1365-2141.1980.tb08712.x>
- Baum, M., R. N. Loeppky, S. Thielen and G. Eisenbrand (2008). Genotoxicity of Glycidamide in Comparison to 3-N-Nitroso-oxazolidin-2-one. *J. Agric. Food Chem.* 56(15): 5989-5993. <https://doi.org/10.1021/jf703741a>
- Benziane A.B., A.D. Bourasb, A. Mezainib, A. Belhadrib and M. Benalia (2019). Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. *Drug. Chem. Toxicol.* 42(2), 157-166. <https://doi.org/10.1080/01480545.2018.1450882>
- Bousalham, R., L. J. Rhazali, A. Harmouch, H. Lotfi, B. Benazzouz, A. E. Hessni, A. Ouichou, O. Akhouayri and A. Mesfioui (2015). Does Argan Oil Supplementation Affect Metabolic Parameters and Behavior in Wistar Rats? *Food. Nutr. Sci.* 6(10): 57389. <https://doi.org/10.4236/fns.2015.69085>
- Can, Ü., F. H. Yerlikaya, Y. Yener and S. Çakır (2016). Effects of High-Fat Diet and Acrylamide on Tissue Oxidant and Antioxidant Levels in Rats. *Selcuk Med. J.* 32(2): 38-42.
- Descamps-Latscha, B. and V. Witko-Sarsat (1999). Neutrophil polymorphonuclear leukocyte and monocyte-macrophage relationships. *Rev. Fr. Allergol. Immunol. Clin.* 39(4): 241-248. [https://doi.org/10.1016/S0335-7457\(99\)80048-3](https://doi.org/10.1016/S0335-7457(99)80048-3)
- Donmez, H. H., N. Donmez, I. Kısadere and I. Undag (2019). Protective effect of quercetin on some hematological parameters in rats exposed to cadmium. *Biotech. Histochem.* 94(5): 381-386. <https://doi.org/10.1080/10520295.2019.1574027>
- Dönmez, H. H., S. Emrah and M. Boydak (2007). Determination of alpha naphthyl acetate esterase activity in peripheral blood leucocytes of Kangal fish (*Garra rufa*). *Eurasian J. Vet. Sci.* 23(2): 81-84.
- Dönmez, H. H. and E. Sur (2008). Hematology and Enzyme Histochemistry of the Peripheral Blood Leucocytes in Rock Partridges (*Alectoris graeca*). *Poult. Sci.* 87(1): 56-60. <https://doi.org/10.3382/ps.2007-00328>
- Dönmez, N., H. H. Dönmez, I. Kısadere and N. Kadiralieva (2016). Some hematological values and alpha-naphthyl acetate esterase (ANAE)-positive lymphocyte ratios in Jaydara sheep. *J. Adv. Vet. Anim. Res.* 3(1): 8-12.
- Eze, E. D., A. M. Afodun, J. Kasolo and K. I. Kasozi (2019). Lycopene improves on basic

- hematological and immunological parameters in diabetes mellitus. *BMC Res. Notes.* 12(1): 805. <https://doi.org/10.1186/s13104-019-4841-8>
- Fang, J., C. L. Liang, X. D. Jia and N. Li (2014). Immunotoxicity of Acrylamide in Female BALB/c Mice. *Biomed. Environ. Sci.* 27(6): 401-409. <https://doi.org/10.3967/bes2014.069>
- Farouk, S. M., F. A. Gad, R. Almeer, M. M. Abdel-Daim and M. A. Emam (2021). Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brain. *Biomed. Pharmacother.* 138: 111458. <https://doi.org/10.1016/j.biopha.2021.111458>
- Ghorbel, I., S. Maktouf, C. Kallel, S. Ellouze Chaabouni, T. Boudawara and N. Zeghal (2015). Disruption of erythrocyte antioxidant defense system, hematological parameters, induction of pro-inflammatory cytokines and DNA damage in liver of co-exposed rats to aluminium and acrylamide. *Chem. Biol. Interact.* 236: 31-40. <https://doi.org/10.1016/j.cbi.2015.04.020>
- Gul, S., M. Ahmad, A. Khan and I. Hussain (2007). Haemato-biochemical observations in apparently healthy equine species. *Pakistan Vet. J.* 27(4): 155-158.
- Guo, J., X. Cao, X. Hu, S. Li and J. Wang (2020). The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats. *BMC Pharmacol. Toxicol.* 21(62): 1-10. <https://doi.org/10.1186/s40360-020-00440-3>
- Hatipoglu, D., M.B. Ates, G. Senturk and A. Bulut (2024). Metabolomic modelling and neuroprotective effects of carvacrol against acrylamide toxicity in rat's brain and sciatic nerve. *Clin. Exp. Pharmacol. Physiol.* 51(3):e13841. doi:10.1111/1440-1681.13841
- Imai, T. and T. Kitahashi (2014). A 13-week toxicity study of acrylamide administered in drinking water to hamsters. *J. Appl. Toxicol.* 34(1): 57-65. <https://doi.org/10.1002/jat.2831>
- Karimi, Z., A. Mirza Alizadeh, J. Ezzati Nazhad Dolatabadi and P. Dehghan (2019). Nigella sativa and its Derivatives as Food Toxicity Protectant Agents. *Adv. Pharm. Bull.* 9(1): 22-37. <https://doi.org/10.15171/apb.2019.004>
- Kavuşan, H. S. and M. Serdaroglu (2019). As a thermal process contaminant acrylamide: formation mechanisms and strategies of reducing acrylamide content in meat products. *TURJAF.* 7(2): 173-185. <https://doi.org/10.24925/turjaf.v7i2.173-185.1944>
- Kisadere, I., M. F. Aydın and H. H. Dönmez (2022). The influence of chitosan oligosaccharide on some hematological parameters in rats exposed to cadmium. *Veterinarski Arhiv.* 92(1): 87-95. <https://doi.org/10.24099/vet.arhiv.1319>
- Lebda, M. A., S. B. Gad and R. R. Rashed (2015). The effect of lipoic acid on acrylamide-induced neuropathy in rats with reference to biochemical, hematological, and behavioral alterations. *Pharm. Biol.* 53(8): 1207-1213. <https://doi.org/10.3109/13880209.2014.970288>
- Mahmood, S. A. F., K. A. M. Amin and S. F. M. Salih (2015). Effect of acrylamide on liver and kidneys in albino wistar rats. *Int. J. Curr. Microbiol. App. Sci.* 4(5): 434-444.
- Mahmood, S. A. F., K. A. M. Amin, H. S. Rahman and H. H. Othman (2016). The pathophysiological effects of acrylamide in Albino Wistar rats. *Int. J. Med. Sci. Public. Health.* 5(7): 42-48.
- Mehri, S., M. Shahi, B. M. Razavi, F. V. Hassani and H. Hosseinzadeh (2014). Neuroprotective effect of thymoquinone in acrylamide-induced neurotoxicity in Wistar rats. *Iran. J. Basic Med. Sci.* 17(12): 1007-1011. <https://doi.org/10.22038/IJBMS.2015.3860>
- Morales, G., M. Jimenez, O. Garcia, M. R. Mendoza and C. I. Beristain (2014). Effect of natural extracts on the formation of acrylamide in fried potatoes. *Food Sci. Technol.* 58(2): 587-593. <https://doi.org/10.1016/j.lwt.2014.03.034>
- Nowak, A., M. Zakłós-Szyda, D. Żyżelewicz, A. Koszucka and I. Motyl (2020). Acrylamide Decreases Cell Viability, and Provides Oxidative Stress, DNA Damage, and Apoptosis in Human Colon Adenocarcinoma Cell Line Caco-2. *Molecules.* 25(2): 368. <https://doi.org/10.3390/molecules25020368>
- Paulsson, B., A. Rannug, A. P. Henderson, B. T. Golding, M. Törnqvist and M. Warholm (2005). In vitro studies of the influence of glutathione transferases and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 580(1): 53-59. <https://doi.org/10.1016/j.mrgentox.2004.11.006>
- Raju, J., J. Roberts, M. Taylor, D. Patry, E. Chomyshyn, D. Caldwell, G. Cooke and R. Mehta (2015). Toxicological effects of short-term dietary acrylamide exposure in male F344 rats. *Environ. Toxicol. Pharmacol.* 39(1): 85-92. <https://doi.org/10.1016/j.etap.2014.11.009>
- Rawi, S. M., M.-A. S. Marie, S. R. Fahmy and S. A. El-Abied (2012). Hazardous effects of acrylamide on immature male and female rats. *African J. Pharmacy and Pharmacology.* 6(18): 1367-1386. <https://doi.org/10.5897/AJPP12.148>
- Rivadeneira-Domínguez, E., Y. Becerra-Contreras, A. Vázquez-Luna, R. Díaz-Sobac and J. F. Rodríguez-Landa (2018). Alterations of blood

- chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. *Tox. Rep.* 5:1124-1128. <https://doi.org/10.1016/j.toxrep.2018.11.006>
- Shrivastava, S., S. K. Nirala, M. S. Reshi, S. Shukla, A. Sharma and C. Uthra (2019). Protective efficacy of vitamin F against acrylamide induced toxicity: studies on oxidative stress biomarkers. *Open Biomark. J.* 9(1): 62-69. <https://doi.org/10.2174/1875318301909010062>
- Şekeroğlu, Z. A., B. Aydın and V. Şekeroğlu (2017). Argan oil reduces oxidative stress, genetic damage and emperipolesis in rats treated with acrylamide. *Biomed. Pharmacother.* 94:873-879. <https://doi.org/10.1016/j.biopha.2017.08.034>
- Tabeshpour, J., S. Mehri, K. Abnous and H. Hosseinzadeh (2019). Neuroprotective Effects of Thymoquinone in Acrylamide-Induced Peripheral Nervous System Toxicity Through MAPKinase and Apoptosis Pathways in Rat. *Neurochem. Res.* 44(5): 1101-1112. <https://doi.org/10.1007/s11064-019-02741-4>
- Tarskikh, M. M. (2006). Damage to erythrocyte membranes as the mechanism for acrylate toxicity. *Bull. Exp. Biol. Med.* 142(6): 690-692. <https://doi.org/10.1007/s10517-006-0452-1>
- Wu, B., X. Chai, A. He, Z. Huang, S. Chen, P. Rao, L. Ke and L. Xiang (2021). Inhibition of acrylamide toxicity in vivo by arginine-glucose maillard reaction products. *Food Chem. Toxicol.* 154:112315. <https://doi.org/10.1016/j.fct.2021.112315>
- Yener, Y., E. Sur, T. Telatar and Y. Oznurlu (2013). The effect of acrylamide on alpha-naphthyl acetate esterase enzyme in blood circulating lymphocytes and gut associated lymphoid tissues in rats. *Exp. Toxicol. Pathol.* 65(1): 143-146. <https://doi.org/10.1016/j.etp.2011.07.002>
- Zaidi, S. I. A., S. Raisuddin, K. P. Singh, A. Jafri, R. Husain, M. M. Husain, S. A. Mall, P. K. Seth and P. K. Ray (1994). Acrylamide Induced Immunosuppression in Rats and Its Modulation by 6-MFA, An Interferon Inducer. *Immunopharmacol. Immunotoxicol.* 16(2): 247-260. <https://doi.org/10.3109/08923979409007093>
- Zamani, E., F. Shaki, S. Abedian-Kenari and M. Shokrzadeh (2017). Acrylamide induces immunotoxicity through reactive oxygen species production and caspase-dependent apoptosis in mice splenocytes via the mitochondria-dependent signaling pathways. *Biomed. Pharmacother.* 94: 523-530. <https://doi.org/10.1016/j.biopha.2017.07.033>
- Zamani, E., M. Shokrzadeh, A. Ziar, S. Abedian-Kenari and F. Shaki (2017). Acrylamide attenuated immune tissues' function via induction of apoptosis and oxidative stress: Protection by l-carnitine. *Hum. Exp. Toxicol.* 37(8): 859-869. <https://doi.org/10.1177/0960327117741753>