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Original Research

Atrazine-Induced Toxicity and Biochemical Changes in *Mus musculus*: the Ameliorative Efficacy of *Nigella sativa* L. (Ranunculaceae) Oil

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Abstract

The study aimed to evaluate the ameliorative potential of *Nigella sativa* oil (N.s) against the adverse effects induced by atrazine (ATZ) in mice. Four groups were divided into: Control group (untreated); N.s group (*N. sativa* oil, 2 mL/kg/BW); ATZ group (atrazine 200 mg/kg/BW); ATZ+N.s group (atrazine 200 mL/kg/BW; and *N. sativa* oil, 2 mL/kg/BW). All doses were administered by gavage once daily for 4 weeks. The atrazine-administered group showed a significant increase in total leukocytes, granulocytes, and agranulocytes and a decrease in red blood cells, hemoglobin, mean corpuscular volume, and packed cell volume. Serum concentrations of aspartate aminotransferase, alanine transaminase, and alkaline phosphatase were high in the ATZ group. Atrazine exposure reduced serum albumin and total protein levels in the blood. A significantly higher concentration of bilirubin, urea, and creatinine was also found in the ATZ group. The simultaneous administration of atrazine and *N. sativa* led to improvements in all the parameters studied above. Thus, this study revealed that atrazine exposure has toxic effects on hematological parameters, liver, and kidney function, and that *N. sativa* oil reduces these toxic effects of atrazine in male mice.

Keywords: *Nigella sativa* oil, atrazine, mice, hematological parameters, liver function test, kidney function test, black seed oil.

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Introduction

Mus musculus (laboratory mouse) is distributed worldwide and has developed into a cosmopolitan rodent species [1]. The laboratory mouse is descended from the house mouse, so its high reproductive rate and adaptability make it a rapidly growing population that can adapt to its environment [1, 2]. This laboratory mouse is often used as a model animal in toxicological and pharmacological research [2]. Herbicides are toxic agrochemicals. They are used to kill weeds in agricultural areas and gardens [3]. Atrazine is a well-known herbicide. It is used to control weeds in crops (asparagus, sorghum, pineapple, and sugar cane) on lawns and golf courses [4]. It is an odorless compound that comes in granular form. It is a synthetic, water-soluble, reactive, flammable, and non-volatile compound with low degradability. Different studies indicate that atrazine residues in surface waters and groundwater cause serious health problems for humans and animals [5-7] have linked serious health problems to atrazine exposure. [8] reported adverse effects of atrazine exposure on the hematology and biochemistry of snow trout. In humans, atrazine exposure can have adverse effects on the reproductive system, causing changes in blood hormones that impair reproductive function. It also causes the formation of tumors and can damage the liver, kidneys, and heart [9]. The results of various studies indicate that pesticides induce lipid peroxidation, which causes harmful biological effects in living organisms. It is known that the administration of atrazine is responsible for the formation of reactive oxygen species (ROS), which are excreted via the kidneys. This leads to atrophy of the glomerulus and vacuolization of the epithelial cells in the juxtamedullary renal tubules. This leads to a reduction in the excretion of creatinine and urea from the body, and the creatinine level in the blood rises [10-12, 13] reported that atrazine exposure at doses of 23, 90, and 360 mg/kg in female mice causes severe toxicity to hematologic parameters and liver and liver function tests with increasing atrazine dose increases. High doses of atrazine significantly reduce the number of lymphocytes, white blood cells, and monocytes. In female mice, it significantly increased the levels of blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Plants are natural sources of therapeutic substances. They are used to promote health and fight different diseases. For centuries, herbal extracts have been used in several indigenous systems of medicine to treat diseases. In recent decades, people's interest in the therapeutic use of natural products has increased as medicinal plants are considered safer compared to modern allopathic treatment [14, 15]. Herbal extracts are commonly used to cure various diseases, such as cancer, diabetes, asthma, and kidney diseases [16, 17].

N. sativa is a dicotyledonous herb and belongs to the family Ranunculaceae. It is native to North Africa, Southern Europe, and Southwest Asia [18, 19]. *N. sativa* is of great importance among Muslims, and it is considered an important remedy. The Prophet Muhammad (peace be upon him) referred to it as a panacea, meaning "universal healer". It is considered a remedy for all diseases except death and the aging process [20]. In the past, several active compounds have been isolated, identified, and reported from different varieties of black cumin. Several studies have reported nutritional components of *N. sativa* seeds, such as vitamins, carbohydrates, minerals, fats, and proteins [21]. The most important active ingredient of *N. sativa* is thymoquinone [22]. Thymoquinone is the main constituent of *N. sativa*, which is thought to have a protective effect on hepatotoxicity and nephrotoxicity as well as reproductive toxicity. Thymoquinone has a strong antioxidant effect, which could protect organs from oxidative damage caused by free radicals. Some studies have shown that thymoquinone and carvacrol have remarkable free radical scavenging properties that prevent tissue damage in organs affected by oxidizing substances in males [23, 24]. [25] reported that in mice receiving oral *Nigella sativa* oil at a dose of 10 mL/kg for 5 days, hematologic parameters and liver function tests improved to normal values compared to X-ray-exposed mice. [26] revealed that mice receiving a hydroalcoholic extract of *N. sativa* seeds orally at doses of 50, 100, and 200 mg/kg for 2 weeks exhibited improved kidney function test parameters, including blood urea nitrogen and creatinine kinase, in male mice. Considering the efficient curative and remedial potential of *N. sativa* and the lack of knowledge on its curative and protective effects against atrazine (herbicide), this study aimed to determine the ameliorative potential of *N. sativa* against atrazine-induced liver, kidney, and hematological toxicity through biochemical analysis.

Material and Methods

Animal Maintenance

The present research was conducted on albino mice (*Mus musculus*) weighing 26–30 gm and 7-8 weeks of age. These were purchased from the University of Veterinary and Animal Sciences, Lahore, Pakistan. Mice were kept in an animal house at the Department of Zoology, University of Sargodha, in stainless steel cages under standard laboratory conditions. These were reared on standard feed (containing wheat, corn, sorghum, barley, rye, triticale, oat, and powder milk in the form of pellets) and drinking water ad libitum.

Chemicals

Atrazine of analytical grade (97%) was purchased from Pak China Chemical Private Limited under the brand name Atratox. *N. sativa* crude oil (Marhaba Laboratories, Pakistan, Lahore) was purchased from the local market. Oil (2 mL/kg) was administered directly into the gullet of the animal through oral gavage.

Experimental Protocol

The study was comprised of four groups, and each group contained 10 male mice. Animals were dissected after four weeks of experimentation.

Group I

Control (untreated): Mice were provided with drinking water and normal feed and kept under observation for four weeks.

Group II

N.s group: Mice were given *N. sativa* oil (2 mL/kg/day) through oral gavage for four weeks.

Group III

ATZ group. Mice were treated with atrazine (200 mg/kg/day mixed in saline) through oral gavage for four weeks.

Group IV

Co-administrated group (ATZ+N.s): Animals were given atrazine (200 mg/kg/day in saline) through gavage, and then after 30 min of atrazine treatment, animals were provided with *N. sativa* oil (2 mL/kg/day) by gavage for four weeks.

Evaluation of Hematological Parameters

At the end of both experiments, the animals were euthanized with cervical dislocation. Blood was collected through direct cardiac puncture and used for evaluation of hematological parameters and biochemical analysis. Blood was kept in EDTA-coated test tubes for examination of hematological parameters (red blood cell (RBC) count, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and differential white blood cells (WBC) count) by the Sysemex KX21N hematology analyzer using a standard protocol. Each sample was run in duplicate.

Evaluation of Serum Biochemical Parameters

Serum was separated from blood by centrifugation at 3000 rpm for 15 min and processed for liver function tests (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, albumin level) and renal functioning tests (urea and creatinine levels). Tests were performed through an automated biochemical analyzer using commercially available kits (Analyticon diagnostic kits) [27].

Statistical Analysis

Results were analyzed through a one-way ANOVA via Pad Prism Version 7.

Results

Comparison of Hematological Parameters Among Experimental Groups

Total Leukocyte Count (TLC) (/µL)

Total leukocyte counts significantly increased in N.s ($p < 0.01$), ATZ ($p < 0.05$), and ATZ+N.s ($p < 0.001$) as compared with the control group. Within treated groups, the highest ($p < 0.05$) leukocyte count was observed in ATZ+N.s. as compared with N.s and ATZ. Which showed the immune-protective potential of *N. sativa* oil against ATZ toxicity in the ATZ+N.s group (Table 1).

Lymphocytes (%)

Lymphocytes (%) significantly increased in N.s ($p < 0.05$), ATZ ($p < 0.001$), and ATZ+N.s ($p < 0.001$) as compared with control. Within the treated groups, the highest lymphocytes (%) were found in ATZ+N.s ($p < 0.001$) and ATZ ($p < 0.05$) as compared with the N.s group. While lymphocyte %age significantly ($p < 0.05$) increased in ATZ+N.s as compared with the ATZ group, which showed *N. sativa* oil enhanced the immune response in ATZ+N.s against ATZ toxicity (Table 1).

Eosinophil (%)

One-way ANOVA showed a significant increase in eosinophil% age in N.s ($p < 0.01$), ATZ ($p < 0.001$), and ATZ+N.s ($p < 0.001$) as compared with control. In treated groups, eosinophils (%) showed a significant increase in ATZ+N.s ($p < 0.01$) and ATZ ($p < 0.05$) as compared to N.s, while no significant change was found among ATZ and ATZ+N.s. (Table 1).

Table 1**.** Leukocyte count of male mice in control and treated male albino mice

Hematological parameters	Control	N.s	ATZ	ATZ+N.s
TLC (/µL)	$4.03 \times 10^3 \pm 0.04$	$4.5 \times 10^3 \pm 0.04^{a**}$	$4.3 \times 10^3 \pm 0.19^{a*}$	$4.68 \times 10^3 \pm 0.04^{a***bc*}$
Lymphocytes %	56 ± 0.47	$61 \pm 0.48^{a*}$	$66 \pm 1.2^{a***b*}$	$68 \pm 1.1^{ab***c*}$
Eosinophil %	1.1 ± 0.16	$2.6 \pm 0.21^{a**}$	$4 \pm 0.36^{a***b*}$	$4.17 \pm 0.33^{a***b**}$
Neutrophil %	47.8 ± 0.854	$53.8 \pm 1.5a^{*}$	$64 \pm 1.08^{ab***}$	$59 \pm 0.70^{a***bc*}$
Monocytes %	3 ± 0.6	3.67 ± 0.33	$6.67 \pm 0.33^{ab***}$	$5.33 \pm 0.34^{ab**c*}$

Note: a = Control versus treated groups (N.s, ATZ, ATZ+N.s), b = N.s versus ATZ, ATZ+N.s, c = ATZ versus ATZ+N.s treated group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM

Neutrophil (%)

The mean value of Neutrophil (%) significantly increased in all treated groups, N.s ($p < 0.05$), ATZ ($p < 0.001$), and ATZ+N.s ($p < 0.001$), as compared with control. While, within the treated groups, a significant increase in neutrophils (%) was found in ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.05$) as compared with the N.s group. Neutrophil (%) significantly decreased ($p < 0.05$) in ATZ+N.s as compared with ATZ, which showed the toxic effect of ATZ persists (Table 1).

Monocytes (%)

Monocytes (%) significantly increased in ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.01$), while N.s showed a non-significant difference as compared with the control. In treated groups, monocytes (%) significantly increased in ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.01$) as compared with the N.s group. While monocytes (%) significantly ($p < 0.05$) decreased in ATZ+N.s as compared to the ATZ-only treated group (Table 1).

Red Blood cell Count (RBC) (/µL)

One-way ANOVA revealed a remarkable ($p < 0.001$) increase in mean red blood cell count in the N.s group, which was reduced ($p < 0.001$) in the ATZ and ATZ+N.s treated groups as compared with the untreated group. Within treated groups, the highest RBC count was observed in the N.s group ($p < 0.001$) as compared with the ATZ and ATZ+N.s treated groups. The highest reduction in RBC count was recorded in the ATZ-treated group, while an increase in RBC's count was observed in the ATZ+N.s group. It indicates the curative effect of *Nigella sativa* against atrazine-generated toxicity (Table 2).

Hemoglobin Concentration (Hb) (g/dl)

Atrazine induced a significant reduction in the mean value of hemoglobin concentration in the ATZ-treated group ($p < 0.01$), while it was significantly ($p < 0.05$) increased in the N.s treated group as compared to the control. Within treated groups, a significant decline in the mean value of hemoglobin concentration was noticed in the atrazine-treated group ($p < 0.001$) as compared with the N.s treated group, while it was significantly ($p < 0.05$) increased in the N.s+ATZ treated animals. The protective effect of *N. sativa* oil against atrazine toxicity was obvious in this parameter (Table 2).

Mean Corpuscular Volume (MCV) (fl)

Atrazine treatment induced a significant reduction in mean corpuscular volume in the ATZ ($p < 0.001$) and N.s+ATZ ($p < 0.05$) treatments, while MCV significantly increased ($p < 0.05$) in the N.s treated group as compared with the control. Among treated groups, the highest value of MCV was found in the N.S. treated group, while a significant decrease ($p < 0.001$) was noticed in the MCV value in the ATZ and ATZ+N.s groups as compared with the N.s group. Black seed oil treatment induced a significant increase in MCV value in N.s+ATZ ($p < 0.01$) as compared with the ATZ group. Which revealed the curative effect of N.s in this parameter (Table 2).

Mean Corpuscular Hemoglobin Concentration (MCH) (pg)

A remarkable increase ($p < 0.05$) in the mean value of corpuscular hemoglobin concentration was noticed in the N.s treated group as compared with the control, while MCH was significantly reduced in the ATZ-exposed group ($p < 0.001$) and ATZ+*N.s* ($p < 0.05$) as compared with the untreated group. Within treated groups, a noteworthy increase in MCH was noticed in the N.s group ($p < 0.05$), which was significantly declined in the ATZ ($p < 0.001$) and ATZ+N.s. ($p < 0.01$) groups. Treatment of *N. sativa* resulted in a remarkable increase in MCH in the ATZ+N.s ($p < 0.001$) group as compared with the ATZ alone-treated group. The protective effect of *N. sativa* oil is clear in this parameter (Table 2).

Packed Cell Volume (PCV) (%)

A significant increase in the mean value of PCV was noticed in the N.s group ($p < 0.01$), but a significant decline in PCV (%) was noticed in the ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.05$) groups as compared with the control. Among the treated groups, the value of PCV (%) was significantly ($p < 0.001$) declined in both ATZ-exposed groups. *Nigella sativa* oil treatment caused a remarkable increase ($p < 0.001$) in PCV (%) as compared with the ATZ-treated group. The protective effect of *N. sativa* oil can be seen in this parameter (Table 2).

Table 2. Blood indices values of male mice in control, N.s, ATZ and ATZ+N.s treated male mice

Hematological parameters	Control	N.s	ATZ	ATZ+N.s
RBC (/µL)	$6.6\times10^6 \pm 0.12$	$7.1\times10^6 \pm 0.04^{a***}$	$5.3\times10^6 \pm 0.05^{ab***}$	$6.0\times10^6 \pm 0.02^{abc***}$
Hb (g/dL)	10.8 ± 0.18	$11.8 \pm 0.24^{a*}$	$8.6 \pm 0.24^{a**b***}$	$9.86 \pm 0.27^{b**c*}$
MCV (fl)	59.6 ± 0.33	$62.7 \pm 0.97^{a*}$	$51.4 \pm 0.34^{ab***}$	$56.2 \pm 0.54^{a*b***c**}$
MCH (pg)	18.6 ± 0.23	$19.4 \pm 0.18^{a*}$	$16.1 \pm 0.08^{ab***}$	$17.8 \pm 0.17^{a*b**c***}$
PCV %	40.7 ± 0.43	$43.3 \pm 0.37^{a**}$	$34.5 \pm 0.42^{ab***}$	$38.5 \pm 0.34^{a*bc***}$

Note: a = Control versus treated groups (N.s, ATZ, ATZ+N.s), b = N.s versus ATZ, ATZ+N.s, c = ATZ versus ATZ+N.s treated group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM

Comparison of Biochemical Parameters Among Experimental Groups

Mean Serum Concentration of AST and ALT (IU/L)

The mean value of AST and ALT increased significantly ($p < 0.001$) in the ATZ and ATZ+N.s groups as compared with the untreated group. Within treated groups, a significant increase ($p < 0.001$) in the mean value of both liver enzymes was noticed in the ATZ and ATZ+N.s groups. Injurious effects of atrazine administration in both ATZ-treated groups were seen, but administration of *N. sativa* oil caused a significant decline ($p < 0.001$) in the mean value of AST and ALT in the ATZ+N.s group as compared with the ATZ-only treated group, which showed the reclamation potential of *N. sativa* oil against atrazine-induced hepatotoxicity (Figure 1A).

Mean Serum Alkaline Phosphate Concentration (ALP) (IU/L)

Statistical data showed that the mean value of alkaline phosphate significantly increased ($p < 0.001$) in the ATZ and ATZ+N.s groups as compared with the control. Among the treated groups, ALP levels increased significantly ($p < 0.001$) in the ATZ and ATZ+N.s groups as compared with the N.s group, while ALP levels significantly ($p < 0.01$) decreased in the ATZ+N.s group as compared with the ATZ group (Figure 1B).

Mean Serum Albumin Concentration (g/dL)

Data analysis showed that albumin concentration decreased significantly in the ATZ ($p < 0.01$) alone treated group, while noteworthy change was not seen in the N.s and ATZ+N.s groups as compared with the untreated group. Among the treated groups, albumin concentration significantly decreased in ATZ ($p < 0.001$) as compared with N.s, while albumin concentration increased in ATZ+N.s ($p < 0.05$) as compared with ATZ (Figure 1C).

Mean Serum Total Protein Concentration (g/dL)

The mean value of total protein in serum showed no significant difference between N.s and ATZ+N.s as compared with the control. Atrazine administration induced a significant decline in the mean value of total protein ($p < 0.01$) as compared with control. While in the treated groups, total protein levels decreased in ATZ ($p < 0.01$). A significant increase in the mean value of total protein was seen in ATZ+N.s ($p < 0.05$) as compared with the ATZ group (Figure 1D).

Mean Serum Bilirubin Concentration (mg/dL)

Data analysis showed that the mean value of bilirubin serum concentration remarkably ($p < 0.001$) increased in the ATZ group as compared with the untreated group. Within treated groups, the highest ($p < 0.001$) increase in the mean value of bilirubin serum concentration was observed in the ATZ alone treated group, while noteworthy change was not seen in the ATZ+N.s and N.s treated groups. *N. sativa* oil treatment induces a significant reduction ($p < 0.01$) in bilirubin concentration in ATZ+N.s as compared with the ATZ group, which proves that *N. sativa* oil can reduce the toxic effect of this parameter (Figure 2A).

Mean Serum Urea Concentration (mg/dL)

One-way ANOVA revealed that a significant increase ($p < 0.001$) in the mean value of urea concentration in the ATZ and N.s.+ATZ treated groups was noticed as a result of atrazine-induced toxicity, while a non-significant change was observed in the N.s group as compared with the untreated group. Among treated groups, the mean value of serum urea concentration significantly increased in both atrazine-exposed groups (ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.01$) as compared with the N.s group. The toxic effect of atrazine is obvious in both ATZ-treated groups, but *N. sativa* oil treatment in ATZ+N.s causes a significant decline in urea mean concentration as compared with ATZ, which proves the ameliorative potential of *N. sativa* oil against atrazine-induced nephrotoxicity (Figure 2B).

Mean Serum Creatinine Concentration (mg/dL)

Data analysis showed that ATZ treatment leads to an increase ($p < 0.001$) in the mean value of serum creatinine concentration in the ATZ and ATZ+N.s groups as compared with the untreated group. Within the treated groups, creatinine levels remarkably increased in the ATZ ($p < 0.001$) and ATZ+N.s ($p < 0.01$) groups as compared with the N.s group. The atrazine toxic effect was clear in the ATZ and ATZ+N.s groups, but to some extent, *N. sativa* oil reduced the atrazine-induced nephrotoxicity by reducing ($p < 0.01$) serum creatinine concentration in the ATZ+N.s group (Figure 2C).

Discussion

Chemicals like pesticides, industrial chemicals, plastics, plasticizers, and pharmaceuticals are well-known endocrine disruptors and toxicants. These chemicals are present in our environment and have an adverse effect on human health [28]. The increased use of pesticides has introduced serious, novel hazards to humans and their livestock [29]. Atrazine is an herbicide. It is used to control or prevent broad-leaf weeds in crops such as corn, sugarcane, etc. In the present study, the atrazine-administered group showed a highly significant reduction in Hb, MCH, MCV, PCV, and RBC counts in the ATZ-treated group as compared with the control. Similarly, [30] reported that atrazine-treated rats at different doses (150 mg/kg, 200 mg/kg, and 300 mg/kg) for 28 days showed a significant ($p< 0.05$) reduction in PCV, RBC, and Hb levels. While [8] reported that atrazine-treated snow trout (at doses of 2-4 ppm) showed a significant reduction in RBC count, MCH, and MCHC, *Nigella sativa* alone treatment induced a significant increase in RBC, Hb, MCV, MCH, and PCV as compared

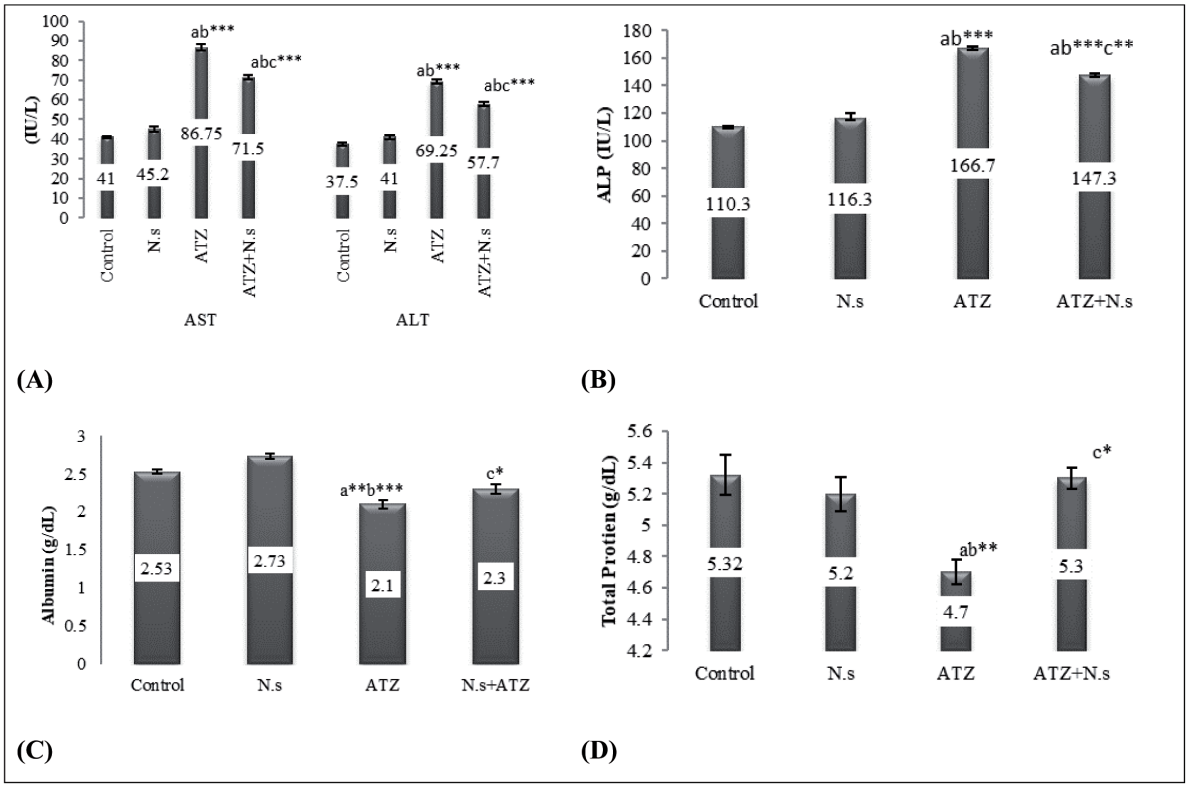

Fig. 1. (A) Mean value of AST and ALT in serum of control, N.s, ATZ and ATZ+N.s groups. (B) Concentration of ALP in control and other treated groups. (C) Mean serum concentration of Albumin in control and other treated groups. (D) Mean serum concentration of total protein in control and other treated groups. a=Control vs. treated groups, b= N.s vs. treated groups, c= ATZ vs. ATZ+N.s treated group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM

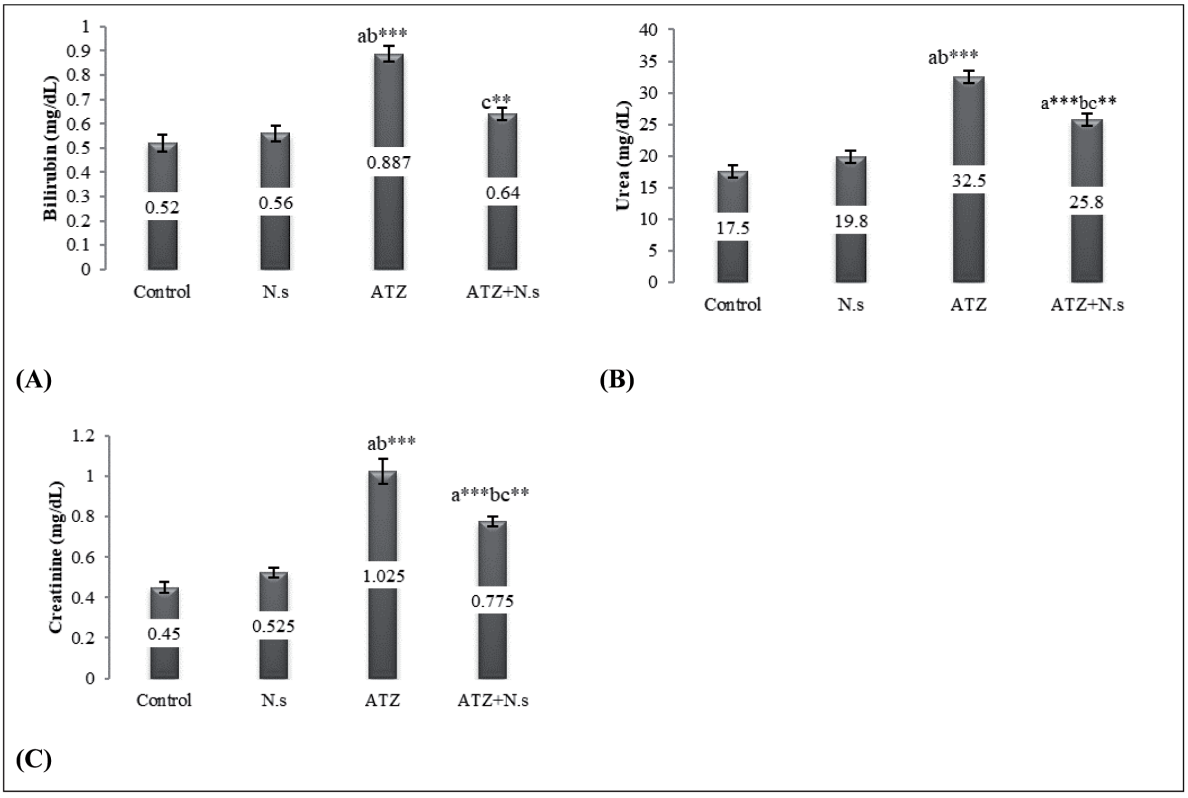

Fig. 2. (A) Mean concentration of serum bilirubin in control and treated male mice. (B) Effect of atrazine alone or in combination with *N. sativa* on serum urea concentration of male mice dose. (C) Effect of atrazine alone or in combination with *N. sativa* on serum creatinine. a=Control vs. treated groups, b= N.s vs. treated groups, c= ATZ vs. ATZ+N.s treated group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM

with the control. These results are in line with [31], who reported that the oral treatment of rats with the fixed oil of *N. sativa* (1 mL/kg/day) for 14 days maintained normal levels of RBC, Hb, and PCV. While co-administration of *N. sativa* with atrazine administration led to an improvement in hematological parameters as compared with only atrazine-treated mice. These results are in line with [32], who investigated that oral administration of *N. sativa* (400 mg/kg) extract for 5 weeks showed protection against carbendazim-induced (150 mg/kg) hematologic, hepatic, and renal toxicities in rats. Similarly, [33] reported that pre-treatment of *N. sativa* oil (2 mL/kg) for five weeks can stimulate the production of erythropoietin and restore the mechanism of erythropoiesis in $AlCl_3$-treated rats. These beneficial effects of *N. sativa* oil are due to its antioxidant activity. In the present study, a significant increase in total leucocyte count was observed in both ATZ-treated groups. White blood cells play an important role in body defense mechanisms. Leukocytosis is a clear indication that the body is taking atrazine as a foreign chemical agent [30] has significantly higher ($p < 0.001$) concentrations of granulocytes (leukocytes, eosinophils, and neutrophils) and agranulocytes (monocytes) than in ATZ-treated mice. The present results are in agreement with [34], who reported that intraperitoneal injection of ATZ (72 mg/kg for 14 days) leads to a significant ($p <0.05$) increase in granulocyte and agranulocyte counts. In the present study, a high lymphocyte count was observed in atrazine-treated animals, which reflects the immunogenic abilities of atrazine. An increase in WBC, granulocytes, and agranulocytes was also found in the present study in the N.s treated group. *Nigella sativa* oil treatment may enhance the immune response. Similarly, [33] reported that rats administered with 2 mL/kg *N. sativa* for 5 weeks significantly ($p <0.01$) increased their WBC count as compared with the control group. The increase in WBC count treated with *N. sativa* oil alone is related to the increase in lymphocyte count, and that is confirmed by [35]. Lymphocyte count was significantly increased in mice treated with *N. sativa* oil alone as compared with the control and other groups of the study, and these results are consistent with [35]. Lymphocytes represent an adaptive immune response. The co-administrated (ATZ+N.s) group showed a significant ($p < 0.05$) increase in TLC, lymphocytes, and monocytes as compared with the ATZ exposed group, which showed that the effects of atrazine were reversed by *N. sativa* co-administration at the cellular immunity level. In several studies, it has been reported that atrazine induces hepatotoxicity, nephrotoxicity, and abnormal changes in hematological parameters [7, 36, 37]. A significantly ($p < 0.001$) higher level of AST, ALT, and ALP was observed in the current study in ATZ groups as compared with the control group. These results are similar to the findings of [38], who reported that 50 mg/kg atrazine administration for 30 days caused a significant elevation in serum concentrations of AST and ALT in rats. These liver enzymes are important in assessing liver injury [39]. Atrazine causes the production of reactive oxygen species that are responsible for the oxidative stress of various organs. Oxidative stress causes lipid peroxidation, which results in hepatic injury [38]. Cellular damage may release these enzymes into circulation. Alanine aminotransferase is considered a more specific enzyme for hepatic injury. It is mainly found in the cytosol of the liver, with low concentrations elsewhere [40]. Hyperbilirubinemia was observed in the present study due to atrazine exposure in both atrazine-administered groups, which supports the above results of hepatic injury. In the present study, *N. sativa* oil alone treatment did not show any notable effect on serum concentrations of AST, ALT, ALP, and bilirubin in N.s and ATZ+N.s groups as compared to the control. Therapy with *N. sativa* oil causes a significant reduction in serum concentrations of liver enzymes (AST, ALT, ALP, and bilirubin) in the co-administered *N. sativa* group (ATZ+N.s) as compared with the ATZ group. These results were in agreement with [41], who reported that *N. sativa* oil therapy (1 mg/kg/day for 30 days) reduced the serum liver enzyme concentration of tramadol-treated rats. [42] reported that thymoquinone (an important constituent of *N. sativa* oil) can antagonize lipid peroxidation and counterpoise the integrity of the cellular membranes of hepatic cells, which causes a reduction in the leakage of liver enzymes. Proteins are the most essential and copious macromolecules in living organisms and play a chief role in the structure and function of the cell and cellular metabolism [43]. In the present study, administration of atrazine caused hypoalbuminemia in the atrazine group as compared with the untreated group. Serum total protein was also significantly reduced in the ATZ group. A decline in total protein content can be considered an index of cellular dysfunction. [44] reported a reduction in serum total proteins in atrazine at doses of 0, 60, 150, and 300 mg administered to male albino rats for 30 days. A reduction in serum total proteins may be due to changes in enzyme activities and impaired protein synthesis by the liver through the production of ROS during the metabolism of cypermethrin. In the present study, *N. sativa* oil alone treatment (N.s group) did not show any notable effect on serum total protein or albumin concentration as compared with the control. Results of the current study revealed that *N. sativa* oil treatment in the co-administered group may induce restoration of the serum total protein and albumin levels, which may cause a significant rise in albumin and serum protein concentration in the ATZ+N.s group as compared with the ATZ group. These results indicate an improvement in the repair mechanisms of the liver and kidney. The present results were consistent with the findings of [45]. They reported the beneficial effect of *N. sativa* on the serum albumin level after infection with *Schistosoma mansoni* in mice. While [46] reported an increase in serum protein concentration by the use of *N. sativa* against paracetamol toxicity in male mice. A high concentration of urea and creatinine was observed in ATZ groups as compared with untreated animals. These results are in agreement with the previous findings of [47]. Their findings suggested that atrazine administration in rats causes an increase in urea

and creatinine concentrations. Atrazine administration causes the generation of reactive oxygen species, which are eliminated through the kidney. It caused atrophy of the glomerulus and the induction of vacuolation in the epithelial cells of the juxtamedullary renal tubules. It leads to a decrease in creatinine and urea clearance from the body, due to which creatinine and urea levels rise in the blood [10, 48]. The serum concentrations of urea and creatinine remain unchanged in the N.s treated group as compared with the control. Administration of *N. sativa* oil in the co-administrated group induced a significant reduction in the concentration of urea and creatinine in the serum of the ATZ+N.s group as compared with the ATZ group. Several studies on *N. sativa* in various models of oxidative stress revealed that most of its pharmacological effects are due to its antioxidant potential. It can scavenge free radicals and ROS. It also inhibits oxidative enzymes, such as cytochrome P450, and prevents lipid peroxidation [49-51] also reported that *N. sativa* oil prevented lipid peroxidation in liposomes and acted as a scavenger of free radicals. In the current study, presumably, the antioxidant action of the *N. sativa* oil prevents the oxidative damage of renal tissues.

Conclusions

It is concluded from the present study that hematological parameters showed that atrazine administration has a profound influence on the blood profiles of the mice. Atrazine exposure may cause anemia, probably due to a decrease in the rate of RBC production or an impairment of erythropoiesis. Atrazine administration induced a toxic effect on the function of the liver and kidney in an animal model. Moreover, the protective effect of *Nigella sativa* crude oil was discerned in almost all parameters of this research. The findings of the present research support the use of crude *N. sativa* oil as a protective and therapeutic agent against atrazine toxicity, to which the human population is intentionally or unintentionally exposed. It is suggested that *Nigella sativa* oil be used and added to the daily routine. More research work is required on its phytochemical analysis and its pharmaceutical components. Thus, it can be used to treat several toxicities caused by several chemicals or diseases. Further, it is suggested to use it for more toxicants or several chemicals that are harmful to one's health. Also, more research is required against atrazine-induced toxicity in male-based models.

Author Contributions

Conceptualization: SYM, M.A, N.A and S.B; Methodology: S.B, F.I, B.A and T.B; Data Curation: M.A, S.Y.M, M.N.K and S.W; Writing original draft preparation: S.Y.M and S.B; Writing-Review and Editing: A.K, M.I, and S.E; Supervision: M.A, and S.B; Funding Acquisition: S.K.A and M.F.E

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Conflict of interest

The authors declare no competing interests.

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