The Journal of Anatomical Sciences
Email:
journalofanatomicalsciences@gmail.com

J. Anat Sci 16(1)

Submitted: September 21st, 2024
Revised: March 14th, 2025
Accepted: March 16th, 2025

Ameliorative Effects of *Nigella sativa* Oil in Hepato-renal Toxicity induced by Cyclophosphamide in Wistar Rats

Murtala Muhammad Jibril[1a], Mohammed Bello Mohammed[2b], Ede Otokpa. Samuel[3c], Ali Ishaq Shugaba[4d], Ekwere Okon Ekwere[2e]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, Sa'adu Zungur University, Bauchi; [2]Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Jos, Jos; [3]Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos; [4]Department of Human Anatomy, Faculty of Basic Medical Sciences, Federal University Lafia, Nigeria

ORCIDs: [a]0000-0002-6170-3194; [b]0000-0002-4069-1956; [c]0000-0002-9972-5698; [d]0009-0001-4925-957X; [e]0000-0002-7508-7492

***Corresponding Author:** Email**:** mjibril09@gmail.com; Tel: 08036377288

ABSTRACT

Cyclophosphamide (CP) is a widely used chemotherapeutic and immunosuppressive agent, however, its clinical applications are often limited by its severe toxicity, particularly affecting the liver and kidneys. The search for natural protective agents against CP-induced toxicity has gained significant attention. *Nigella sativa* oil (NSO), derived from black seeds, has been recognized for its hepatoprotective properties. This study investigated the protective efficacy of NSO against CP-induced hepatorenal toxicity in adult female Wistar rats, by evaluating the potential of NSO in mitigating CP-induced hepatic and renal damage through biochemical, hematological, and histopathological assessments. Twenty-five female Wistar rats were randomly assigned to five groups (n=5): two control groups (positive control received normal saline, negative control received CP) and three treatment groups that received 200 mg/kg, 400 mg/kg, and 800 mg/kg of NSO combined with 0.5 mg/kg CP via the orogastric route for 21 days. Hematological, serum biochemical parameters, organ weights, and tissue histopathology were evaluated. Acute toxicity studies revealed no mortality or adverse effects at 5000 mg/kg NSO. Sub-acute toxicity results showed significant increases ($p < 0.05$) in body and organ weights across all groups. Hematological analysis indicated a dose-dependent increase in white blood cell count. Liver enzymes and urea levels increased in the highest dosage group ($p < 0.05$), in contrast, glucose, total cholesterol and triglycerides decreased significantly. Histopathological examination revealed reduction of necrosis and inflammation. These findings suggest the protective potential of NSO against drug-induced toxicity.

Keywords: *Nigella sativa* oil, hepato-renal, toxicity, cyclophosphamide, Wistar rats

INTRODUCTION

Nigella sativa, known by the common name of black cumin, is an annual flowering plant belonging to the Ranunculaceae family[1]. It is morphologically characterized by a small stem (about 20-30 cm), branched, linear and tapering leaves, delicate flowers colored in white or purplish with 5-10 petals. The fruits are big capsules, constituted by 5-10 units, each containing several tiny black seeds (Figure 1), which are sharp and with a cutting outline[2]. It is native to the Mediterranean Basin and can also be found in Southern Europe, South-Eastern Asia, Northern Africa, Eastern Africa and Northern America[3,4].

Nigella sativa, particularly the seed, is one of the most extensively studied plant species across the world, in terms of the phytochemical properties of its essential oil[5], and the presence of alkaloids, fatty acids, sterols, saponins, tannins, flavonoids, natural organic acids, vitamins and minerals[6,7]. From the pharmacological standpoint, the whole plant is employed in Asia to treat several diseases including headache, infertility, fever, migraine, diabetes, hypertension, inflammations and cancer[8,9,10]. The seed has antimicrobial, analgesic, diuretic, antioxytoxic, antinociceptive, hepatoprotective, cardioprotective and neuroprotective activities [11].

Plant products derived from the barks, flowers, roots, leaves, seeds, fruits are part of phytomedicines[12]. For synthesis of complex chemical compounds, knowledge of the chemical constituents of plants is desirable[13,14]. Phytochemical components such as tannins, carbohydrates, alkaloids, terpenoids, phenolic compounds, steroids and flavonoids are responsible for various pharmacological activities of plants[15-17]. These phytochemical compounds are synthesized through primary or secondary metabolic pathways. The secondary metabolites are taxonomically and chemically diverse compounds, and their complete functions have not been clearly elucidated even though they are extensively used in fields of agriculture, human and veterinary medicines, and related scientific research[18,19]

.

Figure 1: The seeds of Nigella sativa[20].

Nigella sativa seed contains different biochemical constituents with approximately 20 % proteins, 38 % fixed oils, 0.5 – 1.6 % of volatile oils and trace substances such as amino acids, reducing sugar, alkaloids, saponin, crude fiber making about 6.5%, many minerals including calcium, iron, sodium, potassium, copper and zinc[21]. Thymoquinone (TQ, 2-iso-propyl-5-methyl-1, 4-benzoquinone) is identified as the main active compound in NSO, and has been shown to exhibit strong antioxidant properties[22]. This is in addition to its medicinal and other pharmacological properties such as antibacterial, diuretic and antihypertensive[23]. *Nigellone* is another active component present in NSO, and was reported to have antimicrobial effect whereby it could increase the production of interleukin-3 and 1β which has an impact on macrophages[24].

Many researchers were inspired by the numerous uses of *Nigella sativa* in ethnomedicine to isolate

the active components and perform *in vivo* and *in vitro* experiments on laboratory animals and humans in order to understand its pharmacological effect. Immune stimulation, anti-inflammatory, anti-cancer, anti-microbial, anti-parasitic, anti-oxidant, and hypoglycemic effects are only a few examples[25]. The goal of this study was to evaluate the hepato-renal protective actions of NSO following cyclophosphamide-induced liver injury.

MATERIALS AND METHODS

Experimental animals

The study design was approved by the Institutional Animal Care and Use (IACU) Ethical Committee, University of Jos, with approval number: UJ/FPS/F17-00379. A total of forty-five (45) adult female Wistar rats weighing between 120 – 140 g at 6 weeks, were purchased from the Livestock section, School of Veterinary and Medical Laboratory Sciences, National Veterinary Research Institute (NVRI) Vom, Nigeria. All experiments were carried out at the Vivarium of the same Institution. The animals were kept in standard-size plastic cages and maintained under laboratory conditions at normal room temperature, humidity and twelve-hour light and dark cycle. Cleaning of the cages was done daily and the rats were fed with standard diet (Grand Cereal Animal Feeds, Vom) and drinking water *ad libitum*. The rats were acclimatized for two weeks before the commencement of the study. The entire rats received care in compliance with the guidelines of the ethical committee of medical research. Afterwards, they were divided into five groups of five (5) animals each. NSO was administered via oral route with the aid of an orogastric tube.

Plant acquisition and preparation

Nigella sativa seeds were purchased from a commercial shop in Jos metropolis Plateau State, Nigeria. The plant seeds were identified by a taxonomist at the Department of Plant Sciences University of Jos, and authenticated by a Botanist from the Federal School of Forestry, Jos.

It was given herbarium voucher number of 0768. The seeds were prepared for phytochemical and elemental analysis, and Soxhlet method was used for oil extraction (hexane solvent) at the Pharmacognosy Laboratory, Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. Cyclophosphamide (Cycloxan 500 mg), manufactured by Zydus Celexa (Cedila Healthcare Ltd No. 2 ALEAP Industrial Estate, Medical District-500 090 Telengana State, India) was obtained from a patent pharmacy in Jos, Plateau State, Nigeria. Cyclophosphamide was administered at a dosage of 0.5 mg/kg body weight. The average weight of the experimental rats was 120 g (0.12 kg). A stock solution of cyclophosphamide was prepared by dissolving a known quantity of the drug in an appropriate volume of normal saline to achieve the desired concentration. The stock solution concentration was adjusted to ensure precise and uniform dosing. To accurately deliver 0.06 mg per rat, the required volume was calculated based on the prepared stock solution concentration. A calibrated syringe was used to administer the drug via intraperitoneal injection, ensuring consistent dosing across all experimental groups, and ensured that each rat received the intended 0.5 mg/kg dose, maintaining accuracy and reproducibility in the study. The group and doses administrated are summarized in Table 1.

Table 1: Experimental groups (n = 5 per group)

Experimental Groups	Treatment type
Group I	Positive Control (Normal Saline)
Group II	Negative Control (Cyclophosphamide only)
Treatment group III	200 mg/kg NSO + 0.5 mg/kg Cyclophosphamide
Treatment group IV	400 mg/kg NSO + 0.5 mg/kg Cyclophosphamide
Treatment group V	800 mg/kg NSO + 0.5 mg/kg Cyclophosphamide

Statistical analysis

Data were expressed as a mean ± Standard Error in mean (S.E.M), and analyzed using one-way analysis of variance (ANOVA) to evaluate significant difference between groups and the values of $p<0.05$ was considered significant. A Turkey post hoc test was used to evaluate significant difference between groups using Statistical Package for Social Sciences (SPSS) version 22 for windows (IBM Corporation Released, 2012).

RESULTS

Acute toxicity studies

The oral treatment of 5000 mg/kg body weight of NSO to rats resulted in no mortality. The oil extract had no effect on the neurological system, since there was no change in physical movement or convulsion. During the trial, there were no negative changes in breathing patterns, feces, urine, or mucous membranes.

Sub-acute toxicity (repeated dose study)

Body and organ weights: Throughout the investigation, both the control and all treatment groups showed increases in mean body weight (Table 2). Throughout the four-week study period, however, the mean body weight of the control group was substantially higher than that of the treatment groups ($P < 0.05$). In all of the groups, there were no significant changes in relative body weights.

Hematological studies: Table 4 shows the hematological parameters of the experimental animals investigated. In female rats, the white blood cell count was considerably lower (p 0.05) in the control group than in the highest dosage group (800 mg/kg body weight). Other metrics were not significantly different between the groups.

Serum biochemistry: The impact of NSO N-hexane extract on serum biochemical parameters is shown in the Table 5. The treatment group had greater levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and urea (p0.05) than the control group, with the highest dosage group (800 mg/kg body weight) having the highest values. In a dose-related pattern, glucose, total cholesterol, and triglycerides concentrations reduced (p <0.05) across all treatment groups. Total protein, bilirubin, and albumin concentrations in the blood did not change appreciably.

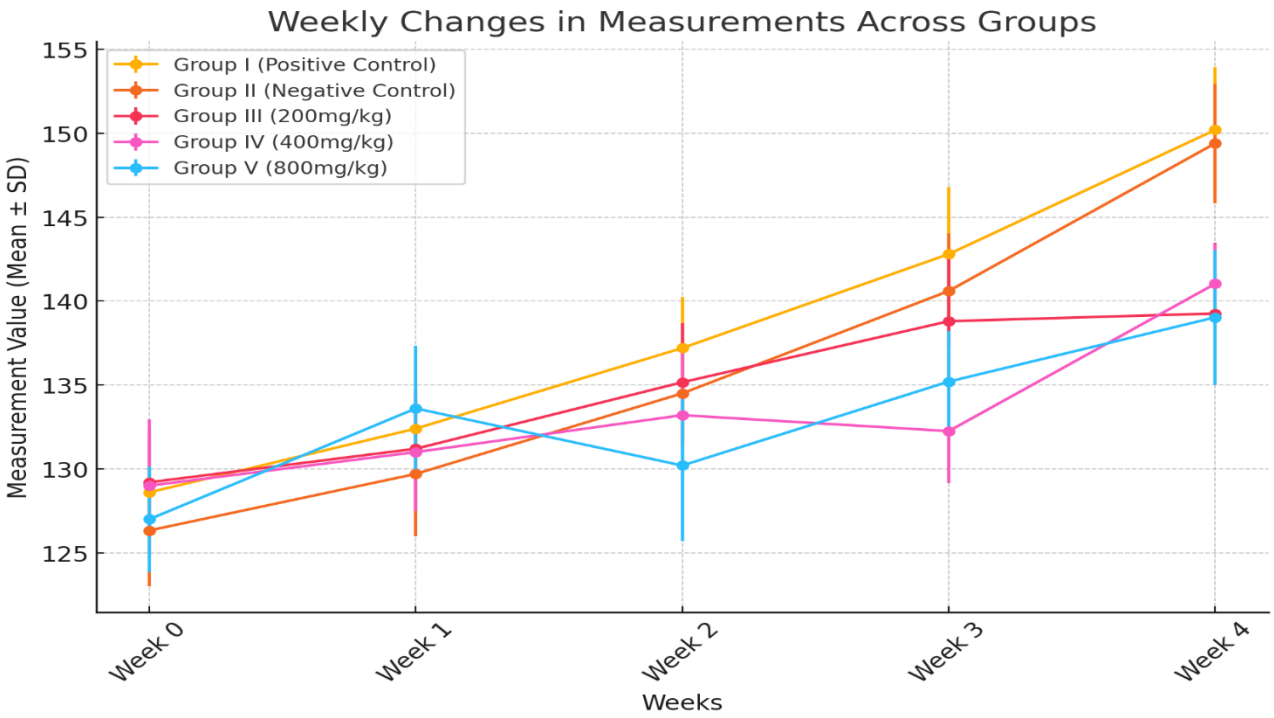

Figure 1: Effect of NSO on body weights of female rats.

Table 2. Effect of NSO on absolute and relative organ weights of female rats

Values expressed as Means ± S.E.M (n=5)

Parameters (g)	Group I (Positive Control)	Group II (Negative Control)	Group II (200 mg/kg)	Group III (400 mg/kg)	Group IV (800 mg/kg)
Liver					
Absolute	3.45 ± 0.28	3.57 ± 0.23	3.37± 0.21	3.64 ± 0.10	3.30 ± 0.35
weight	1.85 ± 0.20	1.73 ± 0.22	1.45± 0.12	1.36 ± 0.10	1.55 ± 0.24
Relative weight					
Kidney					
Absolute	1.88 ± 0.04	1.68 ± 0.16	1.92± 0.06[a]	1.92 ± 0.03	1.86 ± 0.05
weight	0.75 ± 0.03	0.83 ± 0.04	0.60± 0.03	0.61 ± 0.01	0.58 ± 0.04
Relative weight					
Lungs					
Absolute	2.05 ± 0.14	2.03 ± 0.16	2.13± 0.10	2.20 ± 0.11	2.37 ± 0.10
weight	0.90 ± 0.03	0.70 ± 0.33	0.96± 0.05	0.92 ± 0.07[a]	0.99 ± 0.05
Relative weight					
Heart					
Absolute	1.31 ± 0.02	1.40 ± 0.23	1.42 ± 0.04	1.51 ± 0.06	1.70 ± 0.03
weight	0.22 ± 0.01	0.21 ± 0.04	0.24 ± 0.04	0.23 ± 0.04	0.29 ± 0.02
Relative weight					
Spleen					
Absolute	0.81 ± 0.01	0.71 ± 0.06	0.74 ± 0.03	0.71 ± 0.02	0.79 ± 0.01
weight	0.37 ± 0.01	0.34 ± 0.03	0.35 ± 0.01	0.36 ± 0.02	0.37 ± 0.01
Relative weight					
Ovaries					
Absolute	0.21 ± 0.01	0.23 ± 0.04	0.22 ± 0.03	0.20 ± 0.03	0.20 ± 0.02
weight	0.07 ± 0.01	0.05 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Relative weight					

Means that are significantly different are presented in columns with different superscripts
$p < 0.05$. n = number of animals in each group
The superscripts in the table (a, b, and ab) indicate statistical significance between groups.

Table 3 Effect of *NSO* on hematological parameters of Cyclophosphamide (Cy) induced hepatic toxicity female Wistar rats after 21 days administration (n = 5)

Parameters	**Group I (Positive Control)**	**Group II (Negative Control)**	**Group III (200 mg/kg)**	**Group IV (400 mg/kg)**	**Group V (800 mg/kg)**
PCV%	41.25± 0.48	41.35±0.53	39.36±1.37	41.52 ± 1.27	42.18±2.52[b]
RBC ($x10^6/\mu l$)	6.54± 0.42	6.43± 0.35	5.36 ±0.51	6.80 ± 0.22	6.86±0.48
Hb(g/dL)	11.68± 0.38	11.37±0.43	11.45 ±0.26	11.57 ± 0.62	12.47±0.57
MCV (pg)	57.04± 0.42	56.04±0.32	56.10 ±2.34	57.09 ± 2.06	57.46±2.86
MCHC (g/dl)	30.78± 0.40	29.68±0.40	29.52 ±1.68	29.48 ± 0.34	28.47±0.54
Platelets ($x10^3\mu l$)	645.50± 17.46	637.50±2.43	614.86 ± 16.28	628.67 ± 16.02	648.76± 15.62[ab]
WBC ($x\ 10^9/l$)	7.62± 0.48	7.42± 0.45	7.44 ±0.66[b]	7.86 ±0.64[b]	8.06±0.63[a]
Neutrophils (%)	24.04± 1.48	24.12± 1.52	22.34 ±1.62	23.57 ±1.58	24.46±1.86
Eosinophils (%)	2.02± 0.62	2.12± 0.42	2.87 ±0.47	2.60 ± 0.38	2.75±1.88
Basophils (%)	0.00± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00
Lymphocytes (%)	70.46± 1.76	69.43± 1.53	66.80 ±1.78[b]	68.20 ±1.87	70.40±1.68
Monocytes (%)	3.05± 0.58	3.02± 0.56	3.40 ±0.65	3.00 ±0.45	3.25± 0.42

Values are expressed as Mean± S.E.M. The superscripts in the table (a, b, and ab) indicate statistical significance between groups. PCV- packed cell volume, RBC- red blood cell, Hb- hemoglobin, MCV- mean corpuscular volume, MCHC- mean corpuscular hemoglobin concentration, WBC- white blood cell

Table 4 Effect of *NSO* on Biochemical parameters of cyclophosphamide (Cy) induced hepato-toxicity female Wistar rats after 21days administration (n = 5)

Parameters	**Group I (Positive Control)**	**Group II (Negative Control)**	**Group II (200 mg/kg)**	**Group III (400 mg/kg)**	**Group IV (800 mg/kg)**
Alkaline Phosphatase (µ/l)	153.26± 6.07	161.24±5.07	152.68± 4.58^{ab}	175.02± 4.78^{ab}	173.67± 6.18^{a}
Alanine transferase (µ/l)	57.18± 5.82	59.16± 6.72	62.83± 4.55^{ab}	67.25± 5.84^{ab}	75.20± 5.22^{a}
Aspartate transference (µ/l)	162.48± 7.48	160.42± 4.42	158.03± 8.48^{b}	182.58± 5.53^{ab}	232.25± 6.37^{a}
Total protein (g/dl)	6.52± 0.26	6.12± 0.23	6.71± 0.22	6.27± 0.29	6.30± 0.22
Albumin (g/dl)	3.14± 0.07	3.04± 0.03	2.88± 0.09	3.26± 0.17	2.72± 0.15
Direct Bilirubin (mg/dl)	0.08± 0.05	0.02± 0.08	0.13± 0.01	0.11± 0.01	0.14± 0.02
Total Bilirubin (g/dl)	0.18± 0.05	0.18± 0.05	0.25± 0.02	0.17± 0.01	0.20± 0.04
Triglycerides (mg/dl)	102.64± 3.52	104.64± 6.22	61.25± 2.56^{c}	46.00± 2.97^{d}	33.80± 2.71^{e}
Total cholesterol (mg/dl)	38.36± 3.48	38.64± 3.37	38.60± 2.27	39.60± 4.26	35.75± 2.20
Creatinine (mg/dl)	0.53± 0.05	0.56± 0.03	0.48± 0.04	0.63± 0.02	0.58± 0.05
Urea (mg/dl)	24.46± 1.26	24.22± 1.22	24.80± 2.60	25.78± 2.15	26.98± 1.36
Glucose (mg/dl)	108.53± 3.78	102.24± 3.42	96.77±6.75	74.17± 6.76	89.90± 5.99

The superscripts in the table (a, b, and ab) indicate statistical significance between groups.

Histological observation

The liver sections of the experimental groups revealed distinct histopathological variations, which were analyzed based on key parameters such as portal vein congestion, hepatocyte hypertrophy, sinusoidal narrowing, and tissue necrosis. In the positive control (Group 1), liver exhibited normal histological architecture with a well-defined portal vein (PV) and sinusoids (S). There were no evidence of necrosis, congestion, or hepatocyte hypertrophy observed, indicating the absence of hepatic injury. These findings establish a baseline reference for normal hepatic morphology.

The negative control (Group 2): was similar to the negative control. The liver maintained a normal structural appearance with no evidence of congestion or hepatocyte damage. The presence of clear sinusoids and intact hepatocytes suggests that the experimental conditions did not induce hepatic stress in this group (Figure 2).

Rats in Group 3 showed notable congestion of the portal vein. Hepatocyte hypertrophy was evident, leading to narrowing of the sinusoids (S). These changes indicate early signs of hepatic stress and adaptive response to cyclophosphamide toxicity. The presence of minimal necrosis suggests that NS oil at this dose might exert a partial protective effect but insufficient to completely prevent cyclophosphamide -induced hepatotoxicity.

Rats in Group 4 showed severe congestion of the portal vein (PV) was observed compared to Group 3. Increased hepatocyte hypertrophy and sinusoidal narrowing were evident. Focal necrosis (N) was present, indicating progression of cellular damage. These findings suggest that at 400 mg/kg, NS oil does not provide significant hepatoprotection and may not effectively counteract cyclophosphamide -induced oxidative stress.

Group 5 rats had extensive hepatocyte necrosis (N) and tissue distortions were observed, indicating severe hepatotoxicity. Widespread loss of cellular integrity suggests irreversible liver damage. The observed findings indicate that a higher dose of NS oil (800 mg/kg) does not mitigate cyclophosphamide toxicity and may even contribute to hepatic stress, possibly due to pro-oxidant effects at higher concentrations (Figure

2).

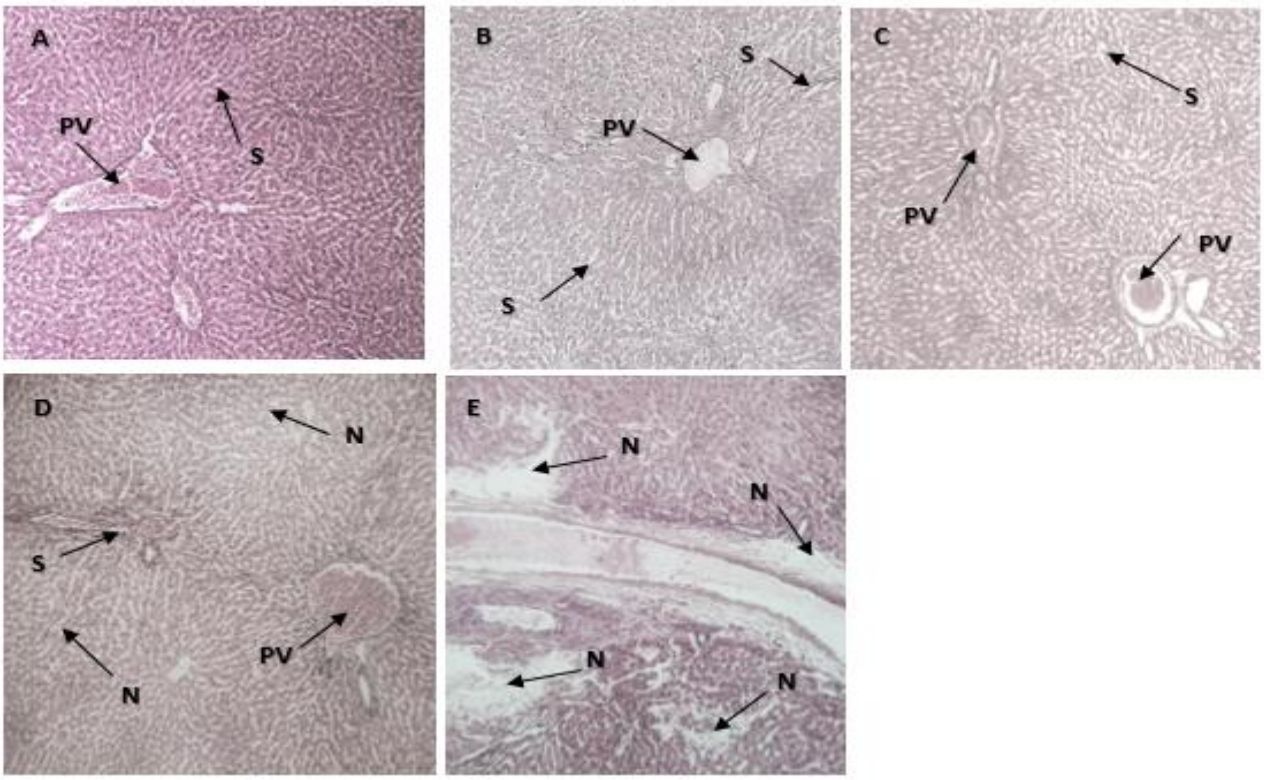

Figure 2: Photomicrograph of the Liver in all experimental groups, exposed to 0.5mg/kg Cyclophosphamide and treated with different concentrations of NS-oil, and the control. H&E Stain, Magnification x100

In Group 1 (A). The liver tissue shows normal appearance with normal central vein (CV) and sinusoids (S). Group 2 (B), There is normal appearance without any congestion in the portal vein, and the hepatocytes. Group 3 (C). Showed congestion of the portal vein (PV), there is also hypertrophy of the hepatocyte resulting in the narrowing of the sinusoids. Group 4 (D). Congestion of the portal vein (PV). There is also hypertrophy of the hepatocytes resulting of narrowing of the sinusoids. Tissue necrosis can be seen across the tissue. Group 5 (E). Severe necrosis of the hepatocytes (N), and tissue distortions are seen across the tissue (Figure 3).

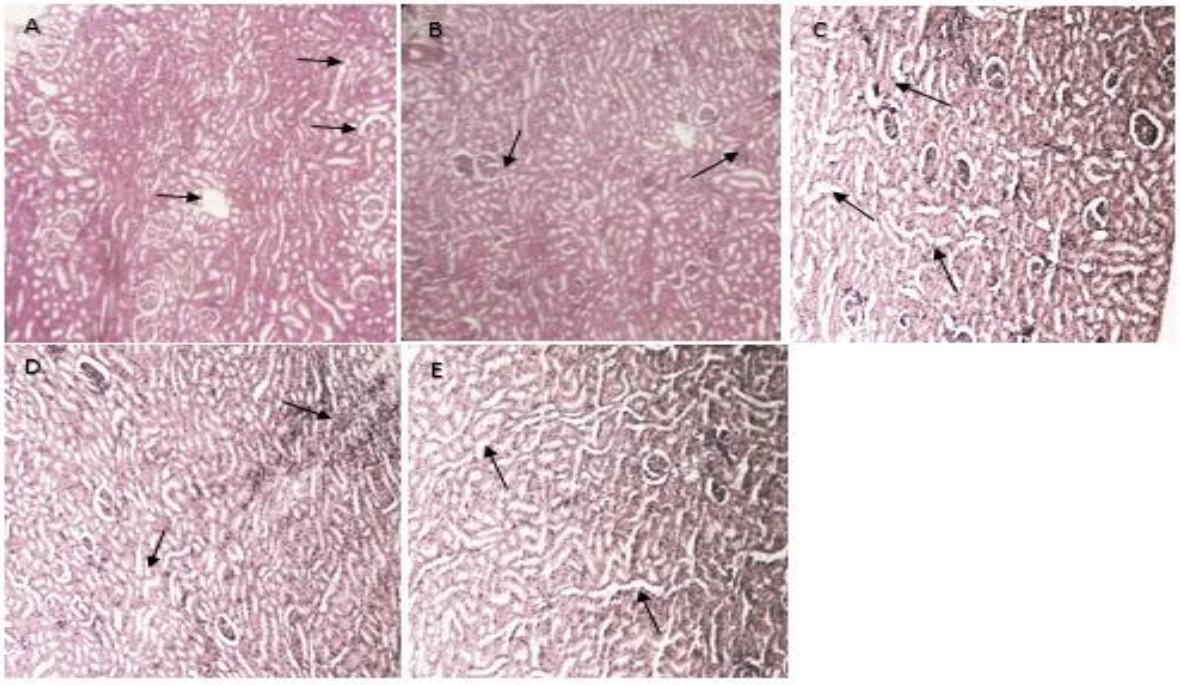

Figure 3: Photomicrographs of rat kidneys from all groups, after exposure to 0.5 mg/kg Cyclophosphamide, and treated with different dose concentration of NS-oil for 21 days. Group 1. (Positive Control). Normal renal corpuscles (black arrows). Group 2. (Negative Control), Group 3. 200 mg/kg NS-oil + 0.5 m/kg Cy. Group 4. 400 mg/kg NS-oil + 0.5 mg/kg Cy. Atrophy of the renal corpuscles. Group 5. 800 mg/kg NS-oil + 0.5 mg/kg Cy. Atrophy of the renal corpuscles, and hypertrophy of the renal medulla. H&E Stain, Magnification x100

Figure 4. Hepatic Adenoma in Wistar rats from experimental groups induced with Cyclophosphamide; the liver appears reddish-brown with a light speckled and rough surface. The arrow shows the liver tumor.

DISCUSSION

The acute toxicity investigation demonstrated that NSO extract was relatively non-toxic, as no treatment-related symptoms of toxicity were observed in the animals during the observation period. A chemical compound has minimal toxicity if the LD_{50} of the substance is 1000 mg/kg body weight [26]. Similarly, according to Lorke's classification [27], a substance with an LD_{50} is between 100 and 1000 mg/kg body weight, is considered to be relatively hazardous. In this study, the LD_{50} of more than 5000 mg/kg body weight indicated that the oil has a wide safety margin, and is consistent with the OECD report that such compounds are relatively non-toxic[28].

The absence of visible adverse effects on the respiratory, neurological, and locomotor systems added to the safety of NSO extract. The use of data based on acute toxicity for clinical reasons is limited by the cumulative effect of drugs at low dosages. Acute toxicity is said to have a low predictive value for determining the toxicity of chemicals in humans and animals [27]. Furthermore, drugs might be damaging without being lethal. Acute toxicity testing, on the other hand, is the first line of defense in determining the safety profile of drugs, including medicinal plants, and its usefulness in determining therapeutic doses. It can also be used to estimate the right doses for another research. Because acute toxicity studies are limited, it is necessary to conduct additional research.

NSO was given orally for 21 days and did not affect mortality. Throughout the study, daily cage-side observations revealed no adverse clinical symptoms, implying that the extract may be safe at the test levels. The weight gain in the extract-treated groups was comparable to the control group. The fact that the rats gained weight every week implies that the extract did not decrease appetite. A large drop in mean body weight has been identified as a key indicator of toxicity [29]. Furthermore, the findings demonstrated that both absolute and relative values were significant. Furthermore, the results demonstrated that oral administration of the extract did not affect both absolute and relative organ weights. When toxicants were supplied that might change either the secretory activities or produce inflammation of the organs, several investigators observed that a rise or decrease in either absolute or relative organ weights reflected organ toxicity [30].

The results indicate that cyclophosphamide induces dose-dependent hepatic damage, manifesting as vascular congestion, hepatocyte hypertrophy, sinusoidal narrowing, and necrosis. While NS oil at lower doses exhibited mild hepatoprotective effects, higher doses were associated with progressive hepatic damage. The observed necrosis and

sinusoidal narrowing in Groups 4 and 5 suggest insufficient antioxidant defense mechanisms at higher doses of NS oil, potentially due to the biphasic nature of thymoquinone (the active compound in NS oil). Previous studies have suggested that low doses of thymoquinone exert antioxidant effects, while higher doses may induce oxidative stress, leading to cytotoxicity.

The health state of an animal can be determined using hematological measures. NSO administered orally at the test dosages for 21 days did not affect hematological parameters in the rats, indicating that there was no interference with red blood cell production. RBCs play a crucial role in the transfer of respiratory gases. The fact that there were no treatment-related impacts on RBC and Hb suggests that the oil extract had no negative impact on the blood's oxygen-carrying capacity or the amount of oxygen given to the tissues.

The mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCV, MCH, and MCHC) are crucial in the diagnosis of anemia [31]. Some medicinal herbs are known to cause red blood cell breakdown, which can lead to anemia [32]. NSO does not appear to have the ability to cause anemia, hence justifying its use in the treatment of drug-related disorders [33]. The increased total WBC count, suggested that the leucocytes' phagocytic function has improved. Platelets play a vital function in hemostasis, and their decline may have a detrimental effect on thrombopoietin levels [34]. The increased platelet counts after 21 days of NSO extract administration suggest that the extract may not create any coagulation issues, but it may have the potential to improve clotting and reduce hemorrhages.

Biochemical parameters are helpful indicators of an organ's functional integrity. Hepatocellular injury is indicated by aminotransferases [35]. Alanine amino transferase (ALT) is present throughout the body, including the liver, kidneys, pancreas, lungs, brain, heart, and skeletal muscles, whereas aspartate amino transferase (AST) is mostly located in the liver [36]. Alanine amino transferase is present in the cytoplasm of hepatic cells, whereas aspartate amino transferase is found in both the cytoplasm and mitochondria. The dose-related increased levels of these liver enzymes seen in this study indicate that NSO extract has not caused hepatocellular damage, this is consistent with studies reported by Zadeh [37], that linked the pharmacological characteristics of *Nigella sativa* to the presence of thymoquinone and its antioxidant qualities [38]. Thymoquinone protects the liver by inhibiting iron-dependent lipid peroxidation, increasing total thiol content and glutathione levels, radical scavenging, increasing the activity of quinone reductase, catalase, superoxide dismutase, and glutathione transferase, inhibiting the activity of free radicals in both cyclooxygenase and lipoxygenase. As a result, the studies emphasize the function of ROS in liver disorders as well as the mechanisms of NSO in liver damage prevention [39, 40]. The current study confirms previous information protective effect of NSO on toxicity in animal models that might be attributed to repeated dosage exposure.

The variations in serum enzymes levels, might have been produced by the oil extracts extended treatment. However, an increase in enzymes may not indicate systemic toxicity. Although amino-transferase levels suggest hepatocellular injury, they do not always indicate the degree of the damage [41]. The enzyme alkaline phosphatase (ALP) is found in abundance in the microvilli of the bile canaliculi and is utilized to check the plasma membrane's integrity [42]. An increased ALP level in the blood is a well-known sign of cholestatic liver disease [43].

The amounts of total protein, albumin, and bilirubin in the blood reflect the condition and degree of hepatic damage. The normal concentration of protein, albumin, and bilirubin found in the current study, implies that the extract did not affect the synthetic and secretory activities of liver, and that the extract is not fully hepatotoxic. Albumin is the plasma protein with the greatest concentration. It

distributes a variety of chemicals, including medications, and protects the tissues from fluid leakage [44]. Hemolytic anemia, biliary obstruction, and hepatic illness are all linked to elevated levels of bilirubin, a result of the 'heme' component of hemoglobin. The amounts of bilirubin measured in this investigation were unchanged, indicating that no erythrocytes were destroyed as a result of the treatment with the oil extract.

The findings might potentially imply that the NS-oil extract had no effect on hepatic cells' ability to excrete bilirubin. Urea molecule produced by protein catabolism and expelled by the kidney, is a by-product of protein catabolism [45]. High protein diets, dehydration, acute bleeding, and shock can all cause elevated urea levels in the blood [46]. The elevated quantity of urea found in this study, especially at high dose levels, suggests that the excretory ability has been compromised in the kidneys. The higher creatinine level was also indicative of potential nephrotoxicity. Increased creatinine levels in the blood are linked to impaired renal excretory function as a result of sickness or a toxic insult [47].

The preserved renal corpuscles and cortex, however, did not suggest nephrotoxicity in the histological results. Flavonoid and tannin have been shown to protect the kidneys from nephrotoxicity [48,49]. Plasma cholesterol and triglyceride levels give insight into lipid metabolism and the risk of atherosclerosis and coronary heart disease [50]. The lower lipid profile indicated that the extract has no lipolytic activity or the potential to cause atherosclerosis. Several plants that include tannins and flavonoids have been shown to reduce blood lipid levels [51]. As highlighted, tannins, flavonoids, alkaloids, and terpenoids were detected in the n-hexane *Nigella sativa* extracted oil in this study.

The considerable reduction in serum glucose concentrations suggests that the extract has a hypoglycemic effect. In the liver and kidneys, gross and histological examinations indicated no abnormalities. There were also minor lesions observed in the liver, and were backed up by an increase in serum enzyme levels, suggesting that there was some hepatic damage. The impact of anesthesia at the sacrifice of the animals may be linked to that, as the same was observed in the rats in both the treatment and control groups.

CONCLUSION

This study highlights the dose-dependent effects of *Nigella sativa* oil in ameliorating cyclophosphamide-induced liver damage, with lower doses offering some protection while higher doses appear to cause more harm. These findings emphasize the need for further research to determine the safest and most effective dose, understand the underlying mechanisms, and explore its potential use in preventing drug-induced organ toxicity.

Acknowledgement

The authors would like to thank Mr. Thomas Yakubu of the Pharmacology Lab. Faculty of Pharmaceutical Sciences, University of Jos, and Mr. Mada Alessa of the Central Diagnostic Laboratory National Veterinary Research Lab (NVRI) Vom, Plateau State, Nigeria.

Funding Information

This research was sponsored by the Tertiary Education Trust (TET) Fund, and Sa'adu Zungur University Bauchi, Bauchi State Nigeria

Conflict of Interest

All authors declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

The research protocols were approved by the Institutional Animal Care and Use (IACU) protocol,

University of Jos, issued by the ethical committee animal experimental unit with approval number: UJ/FPS/F17-00379.

REFERENCES

1. The Angiosperm Phylogeny Group, Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, *et al*. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc. 2016;181(1):1-20. doi: 10.1111/boj.12385.
2. Mozaffarian D. Dietary and policy priorities for cardiovascular disease, diabetes, and obesity: A comprehensive review. Circulation. 2016;133(2):187-225.
3. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: A systematic review. Electron Physician. 2016;8(1):1832-42.
4. Golkar P, Nourbakhsh V. Analysis of genetic diversity and population structure in Nigella sativa L. using agronomic traits and molecular markers (SRAP and SCoT). Ind Crops Prod. 2019; 130:170-8. doi: 10.1016/j.indcrop.2018.12.074.
5. Nergiz C, Ötleş S. Chemical composition of *Nigella sativa* L. seeds. Food Chem. 1993;48(3):259-61. doi: 10.1016/0308-8146(93)90137-5.
6. Gharby S, Harhar H, Guillaume D, Roudani A, Boulbaroud S, Ibrahimi M, *et al*. Chemical investigation of Nigella sativa L. seed oil produced in Morocco. J Saudi Soc Agric Sci. 2015;14(2):172-7. doi: 10.1016/j.jssas.2013.12.001.
7. Taşkin MK, Çalışkan ÖA, Anil H, Abou-Gazar H, Khan IA, Bedir E. Triterpene saponins from *Nigella sativa* L. Turk J Chem. 2005;29(5):561-9.
8. Wray LV Jr, Ferson AE, Fisher SH. Expression of the Bacillus subtilis ureABC operon is controlled by multiple regulatory factors including CodY, GlnR, TnrA, and Spo0H. J Bacteriol. 1997;179(17):5494-501. doi: 10.1128/jb.179.17.5494-5501.1997.
9. Nergiz C, Ötleş S. Chemical composition of *Nigella sativa* L. seeds. Food Chem. 1993;48(3):259-61. doi: 10.1016/0308-8146(93)90137-5.
10. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review. J Ethnopharmacol. 2016; 190:372-86. doi: 10.1016/j.jep.2016.06.061. PMID: 27364039.
11. Craig FW, Lynch JJ, Rosch PJ. A novel psychophysiological therapy for the treatment of hypertension: analysis of a 16-year case study. Altern Ther Health Med. 2001; 104:96-101.
12. Kamalinejad M, Mojab F, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iran J Pharm Res. 2003; 2:77-82.
13. Parekh J, Chanda S. Antibacterial activities of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turk J Biol. 2008;32(1):63-71.
14. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus, stapf* (Lemon grass). J Adv Pharm Technol Res. 2011;2(1):3-8. doi: 10.4103/2231-4040.79796.
15. Abbas S, Ihle P, Köster I, Schubert I. Prevalence and incidence of diagnosed endometriosis and risk of endometriosis in patients with

endometriosis-related symptoms: Findings from a statutory health insurance-based cohort in Germany. Eur J Obstet Gynecol Reprod Biol. 2012;160(1):79-83. doi: 10.1016/j.ejogrb.2011.09.041.

16. Zaman MJ, Patel A, Jan S, Hillis GS, Raju PK, Neal B, *et al*. Socio-economic distribution of cardiovascular risk factors and knowledge in rural India. Int J Epidemiol. 2012;41(5):1302-14. doi: 10.1093/ije/dyr226.

17. Vasu K, Goud JV, Suryam A, Charya MA. Biomolecular and phytochemical analyses of three aquatic angiosperm. African J Microbiol Res. 2009;3(8):418-21.

18. Lingwan M, Pradhan AA, Kushwaha AK, Dar MA, Bhagavatula L, Datta S. Photoprotective role of plant secondary metabolites: Biosynthesis, photoregulation, and prospects of metabolic engineering for enhanced protection under excessive light. Environ Exp Bot. 2023; 209:105300. doi: 10.1016/j.envexpbot.2023.105300.

19. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al*. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-52.

20. Al Mamun A, Nasir NA, Muniady R. Factors affecting the adoption of social media as a business platform: A study among student entrepreneurs in Malaysia. Vision. 2019;23(1):1-11. doi: 10.1177/0972262918821200.

21. Ahmed S, Roy R, Romi I, Hasan M, Khairul M, Bhuiyan H, *et al*. Phytochemical screening, antioxidant and antibacterial activity of some medicinal plants grown in Sylhet region. J Pharm Biol Sci. 2019; 14:26-37.

22. Anwar MA, Al Disi SS, Eid AH. Anti-hypertensive herbs and their mechanisms of action: Part II. Front Pharmacol. 2016; 7:50. doi: 10.3389/fphar.2016.00050.

23. Lavrich KS, Speen AM, Ghio AJ, Bromberg PA, Samet JM, Alexis NE. Macrophages from the upper and lower human respiratory tract are metabolically distinct. Am J Physiol Lung Cell Mol Physiol. 2018;315(5): L752-L764. doi: 10.1152/ajplung.00208.2018.

24. Rodrigues-Diez R, Ballesteros-Martinez C, Moreno-Carriles RM, Nistal F, Diaz Del Campo LS, Cachofeiro V, *et al*. Resolvin D2 prevents vascular remodeling, hypercontractility and endothelial dysfunction in obese hypertensive mice. Through modulation of vascular and proinflammatory factors Biomed Pharmacother. 2024; 174:116564. doi: 10.1016/j.biopha.2024.116564. PMID: 38608525.

25. Gholamnezhad Z, Havakhah S, Boskabady MH. Clinical and experimental effects of Nigella sativa and its constituents on respiratory and allergic disorders. Avicenna J Phytomed. 2019;9(3):195-212.

26. Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol. 2005;5(13-14):1749-70. doi: 10.1016/j.intimp.2005.06.008.

27. Balbaa M, Ashour K, El-Denshary E, Abdallah DM. *Nigella sativa* oil modulates Th1/Th2 and pro/anti-inflammatory cytokine production: A therapeutic potential in asthma. Int J Immunopathol Pharmacol. 2016;29(3):372-85. doi: 10.1177/0394632016658090.

28. Koshak A, Wei L, Koshak E, Wali S, Alamoudi O, Demerdash A, et al. Nigella sativa supplementation improves asthma control and biomarkers: A randomized, double-blind, placebo-controlled trial. Phytother Res. 2017;31(3):403-9.

29. Goreja WG. Black Seed: Nature's Miracle Remedy. New York: Amazing Herbs Press; 2003.

30. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa.* Phytother Res. 2003;17(4):299-305. doi: 10.1002/ptr.1309.

31. Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Anti-inflammatory effects of the Nigella sativa seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford). 2009;11(5):373-81. doi: 10.1111/j.1477-2574.2009.00059. x.

32. Randhawa MA, Alghamdi MS. Anticancer activity of *Nigella sativa* (black seed)—A review. Am J Chin Med. 2011;39(6):1075-91. doi: 10.1142/S0192415X1100941X.

33. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity—the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 2008; 6:495-510.

34. Tauseef M, Sharma R, Reddy R, Verma RK, Krishnan M, Sharma K, *et al.* Hepatoprotective and antioxidant potential of Nigella sativa oil against acetaminophen-induced liver damage in Wistar rats. Biomed Pharmacother. 2021; 138:111421. doi: 10.1016/j.biopha.2021.111421.

35. Farooqi MIH, Srivastava J, Sultan J. Medical efficacy of *Nigella sativa* seeds. Hamdard Med. 1997; 40:40-4.

36. Abukhader MM. Thymoquinone in the clinical treatment of cancer: Fact or fiction? Pharmacogn Rev. 2013;7(14):117-20. doi: 10.4103/0973-7847.120524.

37. Kanter M, Aktas C, Erboga M. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food Chem Toxicol. 2012;50(3-4):719-25. doi: 10.1016/j.fct.2011.11.041.

38. Hannan MA, Rahman MA, Sohag AAM, Uddin MJ, Dash R, Sikder MH, *et al*. Black cumin (Nigella sativa L.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients. 2021;13(6):1784. doi: 10.3390/nu13061784.

39. Sayed-Ahmed MM, Nagi MN. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol. 2007;34(5-6):399-405. doi: 10.1111/j.1440-1681.2007.04599. x.

40. Gilani AH, Jabeen Q, Khan MAU. A review of medicinal uses and pharmacological activities of Nigella sativa. Pak J Biol Sci. 2004;7(4):441-51. doi: 10.3923/pjbs.2004.441.451.

41. Nagi MN, Almakki HA. Thymoquinone supplementation induces quiescence and augments chemotherapy efficacy in breast cancer. Nutr Cancer. 2018;70(4):586-95. doi: 10.1080/01635581.2018.1460674.

42. Ebru U, Burak U, Yusuf S, Arif K, Faruk TH, Ismail K, *et al*. Cardioprotective effects of Nigella sativa oil on cyclosporine A-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol. 2008;103(6):574-80. doi: 10.1111/j.1742-7843.2008.00312. x.

43. Abdel-Daim MM, El-Tawil OS, Aleya L, Barakat W. Thymoquinone and diallyl sulfide protect against acrylamide-induced hepatotoxicity in rats. Environ Sci Pollut Res Int. 2019;26(12):12117-26. doi: 10.1007/s11356-019-04606-2.

44. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation.

Planta Med. 1995;61(1):33-6. doi: 10.1055/s-2006-957994.

45. Alenzi FQ, El-Bolkiny Y, Salem ML. Protective effects of *Nigella sativa* oil and thymoquinone against doxorubicin-induced toxicity. Cancer Cell Int. 2010; 10:9. doi: 10.1186/1475-2867-10-9.

46. Khan MA, Chen HC, Tania M, Zhang DZ. Anticancer activities of Nigella sativa (Black Cumin). Afr J Tradit Complement Altern Med. 2011;8(5S):226-32. doi: 10.4314/ajtcam.v8i5S.10.

47. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al*. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-52. doi: 10.1016/S2221-1691(13)60075-1.

48. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil on gastric secretion and ethanol-induced ulcer in rats. J Ethnopharmacol. 2002;82(2-3):251-8. doi: 10.1016/s0378-8741(02)00189-0.

49. Tariq M, Al-Meshal IA, Parmar NS, Ageel AM, Al-Yahya MA. Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. Am J Chin Med. 1989;17(3-4):277-84. doi: 10.1142/S0192415X89000344.

50. Salama AB, Makboul RM, Ibrahim WM, Shehata AE. Thymoquinone versus metformin in improving metabolic and testicular dysfunction in high-fat diet/streptozotocin-induced diabetic rats. Environ Sci Pollut Res Int. 2022;29(1):924-35. doi: 10.1007/s11356-021-15679-2.

51. Liu Z, Zhang TT, Yu J, Liu YL, Qi SF, Zhao JJ, *et al*. Excess body weight during childhood and adolescence is associated with the risk of multiple sclerosis: a meta-analysis. Neuroepidemiology. 2016;47(2): 103-8.doi: 10.1159/000450854.