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Nigella sativa (black seed) safety: an overview

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Abstract

Nigella sativa (commonly known as black seed or black cumin), from the family Ranunculaceae, is a plant that grows in countries bordering the Mediterranean Sea. This narrative review discusses the toxicological profile reported by short- to long-term studies that examined different extracts and oils of *N. sativa* seeds. Scientific databases including Web of Science, PubMed, Scopus, and Google Scholar were searched using appropriate keywords. LD_{50} for administered *N. sativa* seed fixed oil varied from 28.8 mL/kg to 3,371 mg/kg in mice, while 21 g/kg of aqueous, methanol, and chloroform extracts of *N. sativa* did not lead to any mortality. Subacute toxicity evaluations indicated that aqueous, methanol, and chloroform extracts of *N. sativa* at doses as high as 6 g/kg do not produce toxicity. Investigation of chronic toxicity found that 2 mL/kg of *N. sativa* fixed oil is slightly toxic. Cytotoxicity studies indicated that *N. sativa* chloroform and petroleum ether extracts are more cytotoxic than its other extracts. Although studies that assessed *N. sativa* toxicity generally introduced it as a safe medicinal herb, to draw a more definitive conclusion on its safety, more detailed studies must be conducted.

Keywords: alpha-hederin; carvacrol; 4-cymene; triterpenoid saponin; thymoquinone

Nigella sativa (*N. sativa*, Ranunculaceae family), commonly known as "black seed" or "black cumin," is a flowering plant that grows in countries bordering the Mediterranean Sea, and in Pakistan, India, and Iran [1]. The people of the Middle East and Southeast Asian countries have used *N. sativa* seeds to treat disorders, such as bronchitis, asthma, and inflammatory, infectious, and gastrointestinal diseases, and applied its oil to treat skin diseases such as boils and eczema [2]. *N. sativa* seeds play an important role in the traditional treatment of various diseases, especially fever, chronic headache, migraine, hypertension, and paralysis [3]. Additionally, the extracts of *N. sativa* are traditionally used as a laxative, intestinal antiprotozoal agent, and carminative [4].

In the past 2 decades, various pharmacological or medicinal aspects of *N. sativa* including its antibacterial [5], anticancer [6, 7], anti-inflammatory [8], antioxidant [9, 10], immunomodulatory [11], analgesic [12–14], diuretic [15, 16], antihypertensive [17], antidiabetic [18–20], neuroprotective [16], gastroprotective [21, 22], and hepatoprotective properties [23], have been reported. *N. sativa* has been used in clinical research for neurological disorders [24–26], hypertension [27, 28], hyperlipidemia [29, 30], obesity [31, 32], rheumatoid arthritis [33–35], lung disease [36], thyroid dysfunction [37], hepatitis [38], and male infertility [39].

The herbaceous plant contains fixed oil (FO, 36%–38%), carbohydrates, proteins, fiber, minerals, volatile oil

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(0.2%), essential oil (0.4%–2.5%), alkaloids, coumarins, and saponins [40–42]. The main bioactive components of *N. sativa* seeds are thymoquinone, carvacrol, 4-cymene, pentacyclic triterpenoid saponins (α-hederin), and alkaloids (nigellicine, nigellicimine, nigellicimine-*N*-oxide, and nigellidine) [43, 44]. Thymoquinone is the main and most abundant (27.8%–57.0%) compound of *N. sativa* seeds essential oil, and the other components, such as carvacrol (5.8%–11.6%), 4-cymene (7.1%–15.5%), 4-terpineol (2.0%–6.6%), *t*-anethole (0.25%–2.3%), and longifolene (1.0%–8.0%) exist at lower amounts [45, 46]. Thymoquinone is also the major volatile component (0.72%–21.03%) of *N. sativa* volatile oil and has shown several pharmacological activities related to oxidative stress [7, 20]. **Figure 1** presents the main bioactive components of *N. sativa* [43, 44].

N. sativa seed extracts and its bioactive components are generally regarded as chemicals with low toxicity [43, 47] that have a wide margin of safety [48, 49]. Nevertheless, the common structure of thymoquinone may cause oxidative stress and damage cellular macromolecules (DNA, lipids, and proteins) and signaling pathways, such as extracellular signal-regulated kinase (ERK), protein kinase C (PKC) and Ras; however, it is postulated that coexistence of other components of *N. sativa* reduces thymoquinone toxicity [45, 50–52].

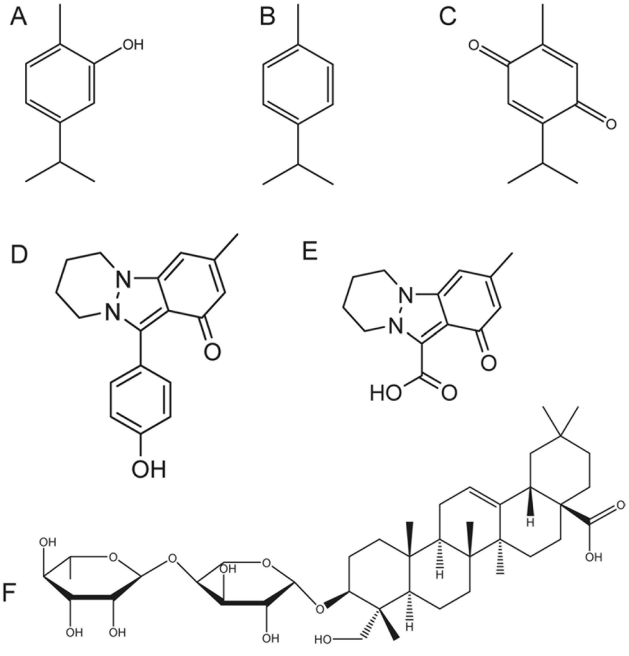

Figure 1. Main bioactive components of *N. sativa*: (A) carvacrol (5-isopropyl-2-methylphenol; National Center for Biotechnology Information. PubChem Database compound identification number [CID] 10364), (B) 4-cymene (4-isopropyltoluene; CID 7463), (C) thymoquinone (4-cymene-2,5-dione; CID 10281), (D) nigellidine (CID 136828302), (E) nigellicine (CID 11402337), and (F) α-hederin (CID 73296).

Figure 2 presents various toxicities observed following exposure to *N. sativa* [49, 53–61].

The present narrative review discusses the toxicity and adverse effects of different extracts and oils obtained from *N. sativa* reported in animals, cell lines, and humans.

Methods

We conducted a literature search of scientific databases including Web of Science, PubMed, Scopus, and Google Scholar. In this search, the following string of keywords was used: "Nigella sativa" or "sativa" or "Nigella" or "black seed" or "black cumin" or "black cumins" or "thymoquinone" or "carvacrol" or "4-cymene" and "safety" or "toxicity" or "cytotoxicity" or "genotoxicity" and "acute" or "subacute" or "subchronic" or "chronic". The wild-card asterisk (*) was used to increase the scope of the search. The reference lists of discovered articles were also reviewed to identify reports that evaluated *N. sativa* toxicity published until March 2020. All studies that assessed the toxicity or reported adverse reactions of *N. sativa* following topical, parenteral, and oral treatment of experimental animals and humans and data from experiments done on cell lines, were included. All included studies were written in English. Review articles were not included.

Discussion

Acute toxicity of *N. sativa* in animal studies

The median lethal dose (50%) (LD_{50}) values of *N. sativa* seed volatile oil, fixed oil (prepared by hexane extraction), and aqueous extract in male Swiss albino (SWR) mice were calculated using a Litchfield and Wilcoxon method. Intraperitoneal administration of volatile oil, aqueous extract, and fixed oil of *N. sativa* has LD_{50} values of 1,853, 3,020, and 3,371 mg/kg, respectively. Based on these data, authors attributed *N. sativa* toxicity to its volatile oil rather than aqueous extract and fixed oil [51].

Oral (10, 15, 20, 25, 30, 40, and 50 mL/kg) or intraperitoneal (0.25, 0.5, 1, 2, 3, 4, and 6 mL/kg) doses of *N. sativa* seeds fixed oil (prepared by hexane extraction) made in gum acacia (5%), were given to male and female mice. Importantly, using thin-layer chromatography (TLC), the fixed oil was characterized and it contained myristic, palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids; triterpenes, and saponosides. For all doses, oral or intraperitoneal administration of *N. sativa* led to behavioral disorders and agitation followed

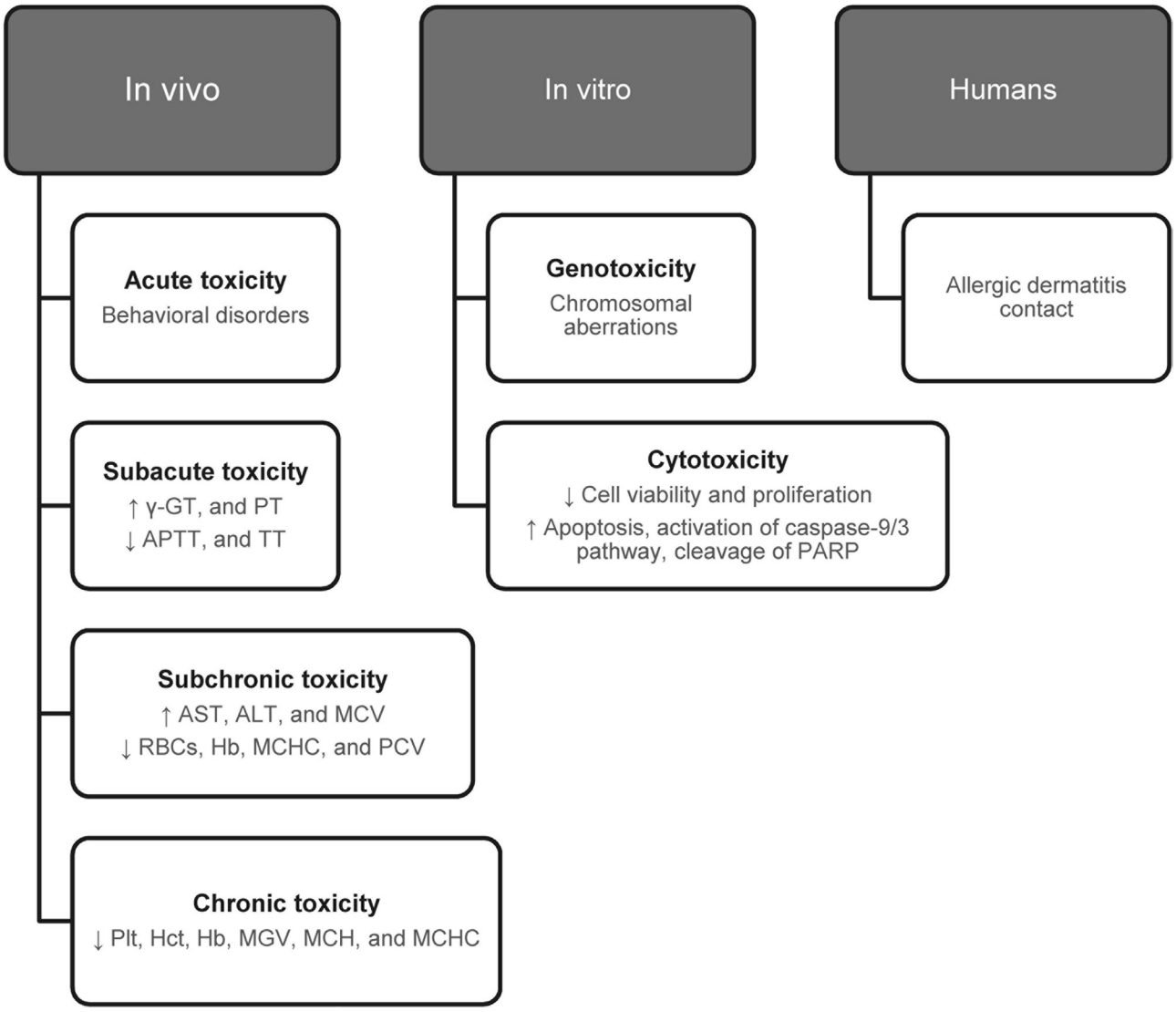

Figure 2. Various toxicities reported for *N. sativa*. *Abbreviations*: γ-GT, gamma-glutamyl transferase; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; PT, prolonged prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; ATIII, antithrombin III; RBCs, red blood cells; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PCV, packed cell volume; MCV, mean corpuscular volume; Plt, platelet; Hct, hematocrit; MGV, mean globular volume; PARP, poly(ADP-ribose) polymerase.

by sedation. Furthermore, 12 h after administration of *N. sativa* (50 mL/kg oral or 4 mL/kg intraperitoneal), all animals died. In surviving mice, activity and growth were rapidly retrieved within 4–8 days after oil administration. This study indicated LD_{50} values of 28.8 mL/kg body weight (bw) and 2.06 mL/kg bw for oral and intraperitoneal FO, respectively, and also indicated that *N. sativa* fixed oil is of low toxicity and possesses a wide margin of safety [49].

LD_{50} values for aqueous, methanol, and chloroform extracts of *N. sativa* seeds following a single oral administration of 4 different doses (6, 9, 14, and 21 g/kg) of the extracts to mice, was evaluated, but zero mortality was observed. The preliminary analysis found neither mortality nor significant morbidity following treatment with the extracts. Therefore, aqueous, methanol, and chloroform extracts were found to be safe and exerted a wide margin of safety following acute exposure [4].

A more recent study reported zero mortality (LD_{50} could not be calculated) following administration of *N. sativa* essential oil at the doses of 0.2, 0.4, 0.4, 0.8, 1 mg/kg to either sex of white Wistar rats and albino mice (NMRI); however, this study lacked a full characterization of the essential oil and only total flavonoid content in terms of quercetin was reported [62].

A summary of studies [4, 49, 51, 62] that examined *N. sativa* acute toxicity is presented in **Table 1**.

Subacute toxicity of *N. sativa* in animal studies

To study subacute toxicity of cumin seeds, 10 mL/kg aqueous extract of *N. sativa* seeds was administered orally to male Sprague Dawley rats for 14 days. In this study, the aqueous extract was not characterized. The results demonstrated a notable increase in gamma-glutamyl transferase (γ-GT) amounts, though serum levels of alkaline phosphatase (ALP) remained unchanged and histopathological examinations did not show any damage; possibly, the findings were affected by an ether anesthesia effect on the release of ALP [63]. Oral administration of 10 mL/kg *N. sativa* seed oil to rats and mice for 48 h caused neither death nor obvious toxicity [53].

Oral administration of 6 g/kg/day of aqueous extract, and methanol and chloroform extracts of *N. sativa* seeds to male mice, for 14 consecutive days, did not affect plasma concentrations of ALP, alanine transaminase (ALT), or aspartate aminotransferase (AST). The pathological findings outlined only minor alterations in mice livers following treatment with these extracts. This study showed that even the 14-day administration of *N. sativa* seeds extracts has a wide margin of safety in mice [4].

Furthermore, 90, 180, 360, and 540 mg/kg of *N. sativa* seed mixed in a powder dough, was given orally for 1, 2, and 4 weeks to normal adult male albino rats. In this study, the authors did not present any information on the chemical composition of the mixture. *N. sativa* at the tested doses transiently altered both anticoagulant and coagulant profiles of rats. In this context, *N. sativa* markedly prolonged prothrombin time caused hyperfibrinogenemia and reduced activated partial thromboplastin time and thrombin time. Nevertheless, antithrombin III (ATIII) and AST levels remained unchanged while ALT and albumin levels significantly increased [54].

Powdered *N. sativa* seeds (0.01, 0.1, and 1 g/kg/day) administered for 28 days to male Sprague Dawley rats, did not affect the bodyweight of the rats, or serum AST and ALT levels compared with a control group. Histological examinations highlighted that 28-days treatment with these doses of *N. sativa* had no harmful impacts on liver tissue in the rats. However, no information on the composition of the seed powder was provided and the doses administered to rats were chosen based on human *N. sativa* consumption (viz., 2 g/d) [64].

Daily administration of *N. sativa* seeds aqueous extract (2, 6.4, 21, 33, and 60 g/kg, orally) to mice for 6 weeks produced hepatotoxic effects (based on histopathological examinations) at doses ≥21 g/kg, but did not influence kidney tissue (in terms of urea and albumin levels). A high mortality rate was found following the administration of 60 g/kg of the extract. The findings highlighted the safety of *N. sativa* at doses lower than 21 g/kg; however, the aqueous extract of the seeds was not characterized [65].

Although for centuries, a black seed and honey mixture (BSH) has used in traditional folk medicine, especially in Islamic countries because of its mention in the Hadith, its toxic effects are unclear. For this reason, a study was conducted to evaluate the effect of 14-day administration of 100, 500, 1,000, and 2,000 mg/kg doses of BSH to male Sprague Dawley rats and found an LD_{50} >2,000 mg/kg. The findings showed no notable alteration in body weight, and differential leukocyte count, or abnormalities in liver and kidney histopathology; however, limitations of this study included a lack of mixture

Table 1. Acute toxicity of *Nigella sativa* in animal studies

Substance/dose	Animals used	Observation period	LD_{50}	Reference
N. sativa seed VO, FO, and AE, i.p.	SWR mice	24 h	AE: 3,020 mg/kg VO: 1,853 mg/kg FO: 3,371 mg/kg	[51]
N. sativa seed FO 0.25, 0.5, 1, 2, 3, 4, and 6 mL/kg, i.p.	Both sexes of mice	15 days	2.06 mL/kg	[49]
N. sativa seed FO 10, 15, 20, 25, 30, 40, and 50 mL/kg, p.o			28.8 mL/kg	
N. sativa seeds AE, ME and CE 6, 9, 14 and 21 g/kg, p.o.	Both sexes of young virgin mice	7 days	No mortality	[4]
N. sativa EO 0.2, 0.4, 0.4, 0.8, 1 mg/kg, p.o.	Both sexes of white Wistar rats and NMRI	Not specified	No mortality	[62]

LD_{50}, lethal dose 50%; VO, volatile oil; FO, fixed oil; EO, essential oil; AE, aqueous extract; ME, methanol extract; CE, chloroform extract; SWR, male Swiss albino; NMRI, albino mice; i.p., intraperitoneal, p.o., per oral.

characterization and histopathological assessment of only 2 organs [66].

Table 2 presents investigations [4, 53, 63–66] conducted to evaluate the subacute toxicity of *N. sativa* in animals.

Subchronic toxicity of *N. sativa* in animal studies

Powdered *N. sativa* seeds (20 and 100 g/kg) were added to the diet of 7-day-old Hibro broiler chicks for 7 weeks. Administration of these doses of *N. sativa* seeds damaged the liver as reflected by increased AST and ALT levels and decreased albumin concentration and cholesterol levels in serum. Furthermore, 100 g/kg of *N. sativa* decreased red blood cells (RBCs), hemoglobin level, mean corpuscular hemoglobin concentration, and packed cell volume despite increases in mean corpuscular volume levels. Together, the results suggested that a 7-week treatment with *N. sativa* seeds did not have a negative influence on growth, pathological features of the main organs, or biochemical/hematological profile of the animals [55].

N. sativa oil obtained from Cairo Chemical Industries Co. (15 and 25 mg/kg) was administered to adult male albino rats for 1 month. However, no information on the chemical composition of the oil was noted. At the end of the experiment, histological changes were found in the cortex of the kidney and to a lesser extent, in hepatocytes in a dose-dependent manner. This study indicated that *N. sativa* oil (oil type not specified) must be used at appropriate doses [48].

Experiments [48, 55] that examined the subchronic toxicity of *N. sativa* are summarized in **Table 3**.

Chronic toxicity of *N. sativa* in animal studies

N. sativa fixed oil (prepared by hexane extraction, 2 mL/kg bw chosen based on the LD_{50} value obtained in an acute study) was orally given to Wistar-Kyoto rats for 12 weeks. Hematological

Table 2. Subacute toxicity of *Nigella sativa* in animal studies

Substance/dose	Animals used	Exposure period	Observation period	Main results	Reference
N. sativa seeds AE 10 mL/kg, p.o.	Male Sprague Dawley rats	14 days	30 days	↑Serum γ-GT and ALT levels. Unchanged serum ALP levels and histopathological examinations	[63]
N. sativa seed oil 10 mL/kg, p.o.	Rats	48 h	Not specified	No toxicity	[53]
	Mice				
N. sativa seeds AE, ME, and CE 6 g/kg, p.o	Both sexes of young virgin mice	14 days	Same 14 days	No toxicity wide margin of safety	[4]
N. sativa powder 90, 180, 360, and 540 mg/kg, locally	Normal adult male albino rats	1, 2, and 4 weeks	Same 4 weeks	Transient alterations in both anticoagulant and coagulant functions	[54]
N. sativa powder 0.01, 0.1, and 1 g/kg/day, p.o	Male Sprague Dawley rats	28 days	Same 28 days	No toxicity	[64]
N. sativa AE 2, 6.4, 21, 33, and 60 g/kg, orally), p.o	*Mus musculus* mice	6 weeks	Same 6 weeks	21 g/kg: hepatotoxic 60 g/kg: high mortality	[65]
BSH 100, 500, 1,000, and 2,000 mg/kg, p.o	Male Sprague Dawley rats	14 days	28 days	No toxicity	[66]

AE, aqueous extract; ME, methanol extract; CE, chloroform extract; γ-GT, gamma-glutamyl transferase; ALP, alkaline phosphatase; ALT, alanine transaminase; BSH, black seed and honey mixture; i.p., intraperitoneal; p.o., per oral.

Table 3. Subchronic toxicity of *Nigella sativa* in animal studies

Substance/dose	Animals used	Exposure period	Observation period	Main results	Reference
N. sativa seeds 20 and 100 g/kg, p.o.	7-day-old Hibro broiler chicks	7 weeks	Same 7 weeks	No toxicity	[55]

p.o., per oral.

examinations showed significant decreases in leukocyte and platelet counts despite significant increases in hematocrit, hemoglobin, mean globular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. No significant alterations were found in AST, ALT, ALP, and GGT levels and no histopathological damages were observed in the heart, kidneys, liver, and pancreas. The researchers suggested that *N. sativa* fixed oil has a wide margin of safety at therapeutic doses, but hematological impacts should be studied further [49].

Oral daily administration of 1 mL/kg bw *N. sativa* seed fixed oil (prepared by hexane extraction) for 12 weeks to Wistar-Kyoto rats led to notable decreases in leukocyte and platelet counts. By contrast, hemoglobin and hematocrit levels were raised significantly. Nevertheless, serum levels of key hepatic enzymes (namely, AST, ALT, ALP, and GGT) did not change significantly relative to controls. Together, these results indicated mild toxicity for *N. sativa* seed FO. In this report, the authors did not specify the sex of the rats or the chemical composition of the fixed oil [56].

Studies [49, 56] that evaluated the chronic toxicity of *N. sativa* in animal models are presented in **Table 4**.

Cytotoxic and genotoxic properties of N. sativa

Various concentrations (0.25, 0.5, and 1 μg/mL) of *N. sativa* oil were tested for possible cytotoxicity in cultures of gingival fibroblasts, but no cytotoxicity of the oil against the fibroblasts was found [67]. This study was limited because it did not indicate which type of oil was examined.

Effects of ethanolic extract of *N. sativa* seeds on rat L6 muscle (L6myc) and human hepatocellular carcinoma (HepG2) cell lines were assessed using a 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. The effect of the extract on glucose transporter-4 (GLUT4) translocation to the plasma membrane of L6-GLUT4 myc cells was examined using an enzyme-linked immunosorbent assay (ELISA) method. The MTT and LDH assays results revealed cytotoxic effects of the extract at doses >500 μg/mL. The median effective concentration (EC_{50}) values for this extract were >2,000 μg/mL against L6myc and >1,470 μg/mL HepG2 cell lines [68].

The influences of various doses of *N. sativa* ethanolic extract (100, 500, and 1,000 μg/mL) on viability and proliferation of nonstimulated and concanavalin A or phytohemagglutinin-stimulated isolated rat splenocytes were examined. Based on the results, 1,000 μg/mL of the ethanolic extract resulted in a marked decrease in cell viability and proliferation in both nonstimulated and stimulated cells [57].

Cytotoxic properties of the essential oil and different extracts (methanol, ether, petroleum ether, chloroform, and water) of *N. sativa* seeds were tested using a brine shrimp lethality assay; results revealed that chloroform and petroleum ether extracts were more toxic than the other extracts [69].

Cytotoxic effects of various concentrations (500, 250, 125, and 62.5 μg/mL) of *N. sativa* seeds volatile oil were tested against 5 human cancer cell lines namely, SCL, SCL-6, SCL-37′6, NUGC-4, and Kato-3, and 3T6 fibroblast line [70].

Cytotoxicity of petroleum ether and aqueous extracts of *N. sativa* for the human acute myeloid leukemia cell line (HL60) was tested, and it was found that petroleum ether extract is more toxic towards these cells having a lower half-maximal inhibitory concentration (IC_{50}) [71]. Treatment of Huh7 human cells with *N. sativa* essential oil (100 mg/mL) did not affect cell viability [72].

N. sativa seed oil and its active component thymoquinone caused a decrease in the size and volume of glioblastoma multiforme (GBM) tumors in a xenograft mouse model. In cell lines including U-1242, U251, U-87, U-373, A172, and SNB19, *N. sativa* seed oil (50–500 μg/mL) and thymoquinone (1–20 μM) inhibited, in a dose-dependent manner cell growth and colony formation in soft agar with an IC_{50} of 260 μg/mL

Table 4. Chronic toxicity of *Nigella sativa* in animal studies

Substance/dose	Animals used	Exposure period	Observation period	Main results	Reference
N. sativa FO 2 mL/kg, p.o.	Wistar-Kyoto rats	12 weeks	Same 12 weeks	↓ Leukocyte and Plt ↑HCT, Hb, MGV, MCH, and MCHC No alteration in AST, ALT, ALP, and GGT levels or histopathological features Slight toxicity	[49]
N. sativa FO 1 mL/kg, p.o.	Wistar-Kyoto rats	12 weeks	Same 12 weeks	↓Leukocyte and Plt ↑HCT, Hb No alteration in AST, ALT, ALP, and GGT levels Slight toxicity	[56]

FO, fixed oil; AST, aspartate aminotransferase; Plt, platelet; Hct, hematocrit; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MGV, mean globular volume; p.o. per oral.

and 6 μM, respectively, activated the caspase-9/3 pathway and induced the cleavage of the death substrate poly(ADP-ribose) polymerase (PARP) [58].

Cytotoxicity, genotoxicity, and antigenotoxicity properties of aqueous extract of *N. sativa* seeds (0.5, 1, 4, 8, 9, and 18 mg/mL) from 3 diverse regions in Morocco were assessed in human C3A cells; the location where the herbs were collected, test system used, and other experimental parameters were important factors influencing the outcomes [73].

Apoptogenic effects of methanolic, n-hexane, and chloroform extracts of *N. sativa* seed were examined in HeLa cancer cells. The findings suggested that all of these extracts can induce apoptosis in cells as confirmed by western blotting, DNA fragmentation tests, and terminal transferase-mediated dUTP-digoxigenin-end labeling (TUNEL) assay [74].

Potential antigenotoxic properties of aqueous extract of *N. sativa* 79.5 μg/mL were tested in F344 hepatocytes treated with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG); results showed that *N. sativa* could not protect hepatocytes from the clastogenic effect of MNNG and produced a remarkable increase of chromosomal aberrations when used as a pretreatment. The findings revealed that this extract displayed a small, but significant, genotoxic potential [59].

Based on the cytotoxic studies, we concluded that the ethanolic extract and aqueous extract of *N. sativa* are safer than other extracts in different cell lines. Essential oil and volatile oil of *N. sativa* are relatively safe.

Table 5 summarizes studies in vitro [57–59, 67–70, 72–74] that evaluated cytotoxicity and genotoxicity of *N. sativa*.

Table 5. Cytotoxic and genotoxic properties of *Nigella sativa* in vitro

Substance	Concentration period	Period	Cell type	Main results	Reference
N. sativa oil	0.25, 0.5, and 1 μg/mL	95 h	Gingival fibroblasts	No cytotoxicity	[67]
N. sativa EE	0–2 mg/mL	24 h	Rat L6 muscle cell line HepG2 cell line	EC_{50} >2,000 μg/mL against L6myc cell lines EC_{50} >1,470 μg/mL against HepG2 cell lines	[68]
N. sativa EE	100, 500, and 1,000 μg/mL	48 h	Isolated rat splenocytes	Cytotoxic effects at 500 and 1,000 μg/mL	[57]
N. sativa EO and ME, PE, CE, AE, and ether extract	10, 100, and 1,000 μg/mL	24 h	BSL	Higher toxicity by chloroform and petroleum ether extracts. LC_{50} petroleum ether: 7 μg/mL LC_{50} chloroform: 21 μg/mL	[69]
N. sativa seed VO	500, 250, 125, and 62.5 μg/mL	Not specified	SCL, SCL-6, SCL-37′6, NUGC-4, Kato-3, 3T6	LC_{50} SCL 155.02 μg/mL LC_{50} SCL-6 185.77 μg/mL LC_{50} SCL-37′6 120.40 μg/mL LC_{50} NUGC-4 384.53 μg/mL LC_{50} 3T6 286.83 μg/mL	[70]
N. sativa PE and AE	670 μg/mL	24 h	HL60	IC_{50} PE 654 μg/mL IC_{50} AE >1,000 μg/ml	[71]
N. sativa EO	100 mg/mL	24 h	Huh7 human cells	No cytotoxicity	[72]
N. sativa seed oil	50–500 μg/mL		U-1242, U251, U-87, U-373, A172 and SNB19	IC_{50} 260 μg/mL	[58]
AE of *N. sativa*	0.5, 1, 4, 8, 9, and 18 mg/mL		Human C3A cells	Positive micronucleus test extract from Setta	[73]
N. sativa seed ME, NE, and CE	2, 2.25, 2.5, 2.75, and 3 μg/mL of methanolic, n-hexane. 0.25, 0.5, 0.75, and 1 ng/mL of chloroform	24 h	HeLa cells	IC_{50} methanolic: 2.28 μg/mL IC_{50} n-hexane 2.20 μg/mL IC_{50} chloroform 0.41 ng/mL	[74]
N. sativa AE	79.5 μg/mL	48 h	F 344 hepatocytes treated with MNNG	↑Chromosomal aberrations	[59]

VO, volatile oil; FO, fixed oil; AE, aqueous extract; ME, methanol extract; CE, chloroform extract; NE, n-hexane extract; EO, essential oil; EE, ethanol extract; PE, petroleum ether extract; EC_{50}, effective concentration; BSL, brine shrimp lethality assay; LC_{50}, concentration that kills 50% of the cells, IC_{50}, half-maximal inhibitory concentration inhibits 50% cell maximal growth; HL60, human myeloid leukemia cell line; MNNG, *N*-methyl-*N*-nitro-*N*-c.

N. sativa toxicity in humans

Administration of *N. sativa* oil to human volunteers at 5 mL/day for 26 days produced no significant hepatic, renal, or gastrointestinal adverse effects [75, 76]. *N. sativa* seeds (3 g/day for 3 months) consumed by 39 centrally obese subjects did not lead to marked side effects [77]. Similarly, diabetic patients who took *N. sativa* seeds (1, 2, and 3 g/day for 3 months) showed no significant alterations in renal or hepatic function [18]. However, epigastric pain and hypoglycemia were observed in hepatitis C virus patients treated with *N. sativa* seed oil capsules [38]. The use of total oil was associated with a marked increase of blood levels of AST and ALT and both the oil and the crushed seeds resulted in significantly increased γ-GT and the ALP activities [78]. *N. sativa* seeds (5 g/day) had an inhibitory effect on CYP2D6 and CYP3A4 in human liver microsomes and healthy human volunteers [79].

A 28-year-old man who had used pure oil of black cumin topically on his neck for 3 months as a treatment of sore throat, presented with a 2-day history of maculopapular eczema, first on the neck and spreading to his arms and back. To our knowledge, this was the first documented case of allergic contact dermatitis caused by topical black cumin oil [60].

A 31-year-old woman with an 8-month history of eczema on both hands and exacerbation of the skin lesions repeatedly applied an ointment containing essential oil extracted from the seeds of black cumin to her palms as a skin-care product. A biopsy from the left hypothenar showed signs of subacute dermatitis. Patch tests showed reactions to the ointment of black cumin in the form of allergic contact dermatitis [61].

A 56-year-old woman presented a 2-day history of severe bullous target-like lesions, compatible with erythema multiform. Histopathology of the lesions indicated lymphocytic infiltration at the dermal–epidermal junction, dermal edema, basal vacuolization, and keratinocyte necrosis. During the last 15 days before the eruption, she had been taking 2 capsules of black cumin essential oil, containing 500 mg of organic *N. sativa* oil (and 7.5 mg of vitamin E) per day. These reactions (presented in **Table 6**), based on previous reports [60, 61, 80], should alert physicians to be informed of potentially intense adverse effects of *N. sativa* essential oils [80].

Clinical trials that examined *N. sativa* and its active constituent, thymoquinone, found these agents safe, but notably several adverse effects such as bloating, nausea, and burning sensation were observed in functionally dyspeptic patients treated with *N. sativa* oil. Moreover, after using *N. sativa* oil and crushed seeds, a slight increase in kidney and liver enzymes was observed [81].

Our literature review to retrieve studies conducted on the short- and long-term toxic effects of *N. sativa* oils and extracts showed that a considerable number of studies were conducted without characterizing the plant material or its extracts; moreover, studies simultaneously in both sexes, in animals other than rats or mice, or performed to provide a full toxicological screening of all parameters of importance, are lacking. Dose ranges vary among studies making it difficult to draw conclusions.

Conclusions

The essential oil obtained from *N. sativa* was found to be safer than the volatile oil against different cell lines. Human case reports indicated allergic contact dermatitis following the use of some preparations containing *N. sativa*. However, clinical trials did not report any severe adverse effects following consumption *N. sativa*. Studies that assessed *N. sativa* safety generally introduced this plant as a safe medicinal herb. Nevertheless, more detailed investigations are still required to have a clearer insight into the toxicological profile of *N. sativa*.

Table 6. *Nigella sativa* toxicity in humans

Substance	Concentration	Exposure route/period	Case history	Main results	Reference
Pure oil of black cumin	Not specified	Used on the neck for 3 months	28-year-old man for treatment of sore throat	Allergic contact dermatitis	[60]
EO of the seeds of black cumin	Not specified	Repeatedly applied as an ointment	31-year-old woman with an 8-month history of eczema on both hands	Allergic contact dermatitis	[61]
Capsules of BCEO	Containing 500 mg of organic *N. sativa* oil and 7.5 mg of vitamin E	Daily 15 days	56-year-old woman	Severe bullous target-like lesions, compatible with erythema multiforme	[80]

EO, essential oil.

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