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Safety Assessment of Black Cumin (nigellasatival) Seeds as a Food Ingredient

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Abstract

The food ingredient known in Western culture as “black cumin” or “black caraway”, Nigella sativa L., has a three-millennial history of use in Middle- and Far-Eastern cultures as a food ingredient and therapeutic agent. Quantitative analysis of the volatile fraction yields widely variable results which may be due to one or a combination of different crop origins or possible varietal differences, contamination, method of extraction, stage of maturation of seed and other factors. Nonetheless, many publications cite quantifiable outcomes in acute, sub-chronic and chronic testing; as well as cytotoxic, mutagenic, and anti-mutagenic effects. There are a few reports describing allergic reactions in humans when N. sativa extracts are applied to the skin. Inasmuch as there is a paucity of credible evidence characterizing the safety of comparable N. sativa seeds or their extracts, there is likewise no evidence that the intact seed of N. sativa is harmful to the public when it is used in food as currently practiced. Notwithstanding the preceding and to paraphrase the Select Committee on GRAS Substances (SCOGS), it is not possible to determine, without additional data, whether a significant increase in consumption [of the whole seed] would constitute a dietary hazard (FDA, 2018).

Introduction

The seed of Nigella sativa L., a member of the Ranunculaceae (Buttercup family), is black or dark brown in color and has a distinctive angular or funnel-shape, with a slightly bitter nutty-peppery taste and strong aroma (Sharma and Longvah, 2021). The seeds of N. sativa are also referred to “black cumin” or “black caraway” and are sometimes confused with the seeds of Cuminum cyminum L. (“true” cumin), Carum carvi L. (“true” caraway), or Bunium persicum (Boiss.) B. Fedtsch (also, Caraum persicum), probably as the result of similarities in physical appearance, confusion regarding common names and opportunities for substitution. There is confusion even with other species of Nigella, such as N. damascene [1].

“True” cumin is derived from the seeds (actually, fruits) of C. cyminum L., a member of the Apiaceae or Umbelliferae family (the celery, carrot, and parsley family). C. cyminum seeds have a distinctive aromatic odor and a spicy, faintly pungent flavor. The seeds of C. cyminum are found in a pod, as are the seeds in N. sativa plants. Both black cumin and true cumin are used in several cuisines, from Indian food to chili, but C. cyminum seeds elicit a slightly hotter taste than N. sativa seeds. Both C. cyminum and N. sativa seeds are also used as therapeutic agents. N. sativa is sometimes also called black caraway to distinguish it from true caraway which is derived from Carum carvi L., another member of the parsley family with achenes (seed-like bodies) on the fruit (like strawberries). True caraway is used for largely the same purposes as N. sativa, as a therapeutic agent and food ingredient but the former having an anise or licorice-type flavor. A fourth plant, Bunium persicum (Boiss.) B. Fedtsch, also a member of the Apiaceae family, is variously called wild caraway, great pignut, black zire, black caraway, carum carvi, Persian cumin, wild cumin or Zireh kuhi (in Iran), among other names. The seeds (actually, “partial fruits”) of B. persicum could, by some, be considered similar to N. sativa, being brown or dark brown, comma-shaped and imparting a smoky-earthy taste. The B. persicum seeds are used in the same manner as the other species named above as spices in foods such as bread, rice, yogurt, cheese, and confectionaries; similar therapeutic applications are also reported in the literature. All four of these plants are grown in relatively the same geographic area, have similar uses and are roughly similar enough in appearance to confuse early travelers from foreign lands, factors which may have perpetuated a corrupted nomenclature. Be that as it may, this monograph will focus on the seeds of Nigella sativa L (N. sativa), to distinguish it from other members of the genus, such as Nigella damascene [2].

N. sativa L. (seed), is steeped in a rich historical and religious background. The plant, from which the seed is obtained, is native to Middle Europe (Bulgaria, Cyprus, and Romania) and part of the Near East (Iran, Iraq and Turkey) and is extensively cultivated from Morocco to northern India and Bangladesh, China, Pacific-facing nations, East Africa, and Russia for use as a spice. N. sativa is also grown on a minor scale for medicinal purposes in North America, Europe, and Southeast Asia. Much of the bioactive property of N. sativa seed is attributed to the presence of thymoquinone, a constituent of the essential oil (Khader and Eckl, 2014; Dubey et al., 2016). As black cumin, N. sativa is approved for food use by the US Food and Drug Administration (FDA). N. sativa has also been determined Generally Recognized as Safe (GRAS) by the Flavor and Extract Manufacturers Association (FEMA), both as black cumin and black caraway (although the seed is not specifically designated). The European Food Safety Authority (EFSA) regards the essential

oil as a "chemical of concern" because of the presence of certain alkaloids.

This review evaluates the safety-in-use of N. sativa L., as distinguished from other members of the genus Nigella, such as Nigella damascene. Further, it is only the seed, obtained from a pod grown by N. sativa, that is discussed herein; no information was located describing consumption of any other part of the plant [3].

Historical perspective and present status

N. sativa seeds have been in use by humans for more than 3,000 years and although its native range is Eastern Europe and part of the Near East, N. sativa is now widely cultivated throughout much of the world. In addition to its use as a food ingredient, N. sativa has been used for centuries in the treatment of a plethora of aliments and is an important drug in Indian Ayurvedic, Unani and Siddha medicines. It is suggested as a remedy of all diseases in one of the Prophetic Hadith and is recommended in Tibb-e-Nabawi (Prophetic Medicine) of the Muslim faith. N. sativa is described as "curative black cumin" and was referred to in Greek (i.e., Hippocratic) medicine as melanthion (literally: little black seed) and, by Dioscorides and Pliny. Avicenna refers to N. sativa seeds as able to "stimulate the body's energy and help recovery from fatigue and dispiritedness". Likewise, there is a reference in in the Old Testament (Isaiah 28:27) i.e., "Caraway is not threshed with a sledge, nor is the wheel of a cart rolled over cumin; caraway is beaten out with a rod and cumin with a stick".

Presently, the major producers of cumin are small and marginal farmers and, is often double-cropped (grown in the same fields) with Nigella damascena. The major producing countries of N. sativa are listed inTable1, and the major trading countries are Egypt, India, Pakistan, Iran, Syria and Turkey. The global consumption of cumin is estimated to be 187,000 metric tonnes (206,132 US tons) and the major importers are Brazil, Canada, Colombia, European Union, Ecuador, Japan, Malaysia, Mexico, South Africa, and the USA [4].

Table1: Major producing countries of cumina.

Country	Production (MetricTonnes)	Production (US tons)
India	250,000	275,577
Syria	10,000	11,023
Turkey	8,000	8,818
Iran	7,000	7,716
China	5,000	5,511
Afghanistan	4,000	4,409

Etymology, taxonomy and description

The common name, genus and name in commerce is Nigella, from modern Latin, the feminine of Latin nigellus, a diminutive of niger or black (referring to the color of the seed, not the flower, which may be white, yellow, pink, pale blue or pale purple). The species name, sativa, is a derivative of the Latin botanical adjective serere (to sow, to plant, to cultivate) or sativum, meaning "cultivated", which is not surprising because it has been cultivated for thousands of years.

The taxonomic classification of N. sativa is provided in Table2. Nigella is in the Ranunculaceae (Buttercup) family. There is disagreement on whether Nigella should be a single genus or be divided into three genera (i.e., Komaroffia Kuntze, Garidella L. and Nigella L.); however, if considered to be a single genus, Nigella, there are 90 proposed species, but only 18 of which are accepted as such and the status of the remainder are "unresolved". Miscellaneous descriptors, classifications and non-medical uses with referencing authority are provided Table4.

Table2: Classification of Nigella sativa L. (Black cumin).

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Magnoliidae
Order	Ranuncuales
Family	Ranunculaceae (Buttercup family)

Genus	Nigella L. (nigella)
Species	Nigella sativa L. (black cumin)

Table3: Recognized species of the genus Nigella (The Plant List, 2012).

N. arvensis L.	N. orientalis L.
N. carpathia Strid.	N. oxypetala Boiss.
N. damascene L.	N. papillosa G. Lopez
N. degenii Vierh.	N. sativa L.
N. deserti Boiss.	N. segetalis M. Bieb
N. fumariifloa Kotschy	N. stricta Strid
N. hispanica L.	N. unguicularis (Poir.) Spenn.
N. nigellastrum (L.) Willk	

Ahmad et al. (2013) describes N. sativa as follows:

N. sativa is a hermaphroditic annual flowering plant which grows from 20 – 90 cm tall, with finely divided leaves 2.5 – 5.0 cm in length, linear to lanceolate in shape; the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5 – 10 petals; the flower terminates the main shoot and ends with flowers on the lowermost branches. The male phase is initiated a few days before the stigmas become receptive; however, the maturing styles often become twisted around the last dehiscing anthers, which results in self-pollination. Fertilized flowers develop into 2- to 3 cm/long capsules with numerous seeds, developing into a follicle after pollination with a single fruit (or pod) partially connected to form a capsule like structure. The fruit is a large and inflated capsule composed of 3 – 7 united follicles, each containing numerous seeds [5].

Macroscopically, N. sativa seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5 mm x 1-2 mm, black externally and white inside, with a slightly aromatic odor and bitter taste. Although Ipor and Oyen claim that "[t]he odor of crushed black cumin seed is reminiscent of lemon with a suggestion of carrot; the taste is strong, pungent and peppery, but also aromatic and nutty [but] to others it resembles oregano". According to Margout, the seeds of N. sativa and a related species, Nigella damascena, are superficially similar to the naked eye; although the seeds of N. sativa are pear-shaped with one flat side and one convex side, whereas the seeds of N. damascena are oval and both sides are curved.

Paarakh (2010) and Margout have similar descriptions of N. sativa at the microscopic level as follows:

Microscopically, a transverse section of the N. sativa seed shows a single layered epidermis consisting of elliptical, thick-walled cells, covered externally by a papillose cuticle and filled with dark brown contents. The epidermis is followed by 2 – 4 layers of thick walled tangentially elongated cells. The endosperm consists of thin-walled cells filled with oil globules.

According to Margout. Microscopically, the seeds of N. damascena are said to be distinctive and different from N. sativa.

Margout compared the taste of the two species (N. sativa vs. N. damascena): while both have a metallic taste when the seed contacts dental enamel and after crushing by the teeth, the N. sativa has the taste of lead pencil, followed by a sharp, aromatic peppery taste, becoming irritant at the base of the throat and leaving a very persistent bitterness on the palate. Whereas the taste of N. damascena after crushing with the teeth, has a persistent taste of dried apple, described as a strong aromatic taste which fills the mouth and persists on the palate [6].

According to Jansen N. damascena is easily distinguished from N. sativa by its leaf-like involucre around flower and fruit. Because the flower of this plant is hidden by the involucre, the British call it: 'love-in-the-mist'.

Margout also notes that the seeds of the two species may be readily distinguished by the GC-MS profiles of their volatile fractions: (1) The alkaloid damascenine is present in N. damascena, but absent from N. sativa and, (2) the N. damascena volatile extract does not contain thymoquinone, whereas this substance is characteristic of N. sativa.

The seeds of N. sativa should be stored in air-tight packages to avoid loss of volatiles and to avoid escaping volatiles contamination of other spices, due to its over-bearing flavor and aroma.

Table4: Authoritative descriptors of Nigella sativa L.

Parameter	Characteristic	Referencing Authority
Botanical source	Nigella sativa L.	FDA, 2021
Standard of Identity	There is no standard of identity for spices.	FDA, 1980
Synonyms	Black cumin, black caraway, Nigelle, nutmeg flower, Roman coriander	FDA, 2021
	Russian caraway, black caraway, Damas black cumin, Nigella sativa L., Nigella damascene L.	Codex Alimentarius Commission, 2017
	Fennel flower	Jansen, 1981
	Nigella cretica Mill.	The Plant List, 2012b; POWO, 2021 (see also previous table)
	Nigella indica Roxb.	
	Nigella truncate Viv.	
	Kalonji (Hindi)	Dubey et al., 2016
	Habbat Albarakah, Alhabahat, Alsawda, and Alkamoun Alaswad (among Muslim communities)	Sohail et al., 2020
	Kalonji, black onion seeds, black cumin seeds, black caraway seeds	European Spice Association, 2018
Functionality in food	Flavoring agent or adjuvant	FDA, 2021
CASNa	977017-84-7b	FDA, 2021
FEMAc (Caraway, black, Nigella sativa L.)	2237 (CASN 977017-84-7)d	Hall and Oser, 1965
FEMA (Cumin, black, Nigella sativa L.)	2342 (CASN 84775-51-9)d,e	Hall and Oser, 1965
Spices and other natural seasonings and flavorings	Caraway, black (black cumin)	AAFCO, 2021
Food additive regulation	21 CFR § 182.10 (Spices and other natural seasonings and flavorings)	
Chemical of concern	Isoquinoline alkaloids, e.g. nigelimine	EFSA, 2012
Novel food status (as black cumin)	This product was on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997. Thus, its access to the market is not subject to the Novel Food Regulation (EU) 2015/2283. However, other specific legislation may restrict the placing on the market of this product as a food or food ingredient in some Member States. Therefore, it is recommended to check with the national competent authorities.	EFSA, 2021
Codex category designation	Group 028 Spices, Class A, type 5, Group Letter Code HS, Subgroup 028A Seeds, Code No. HS 3285 Black caraway, Nigella sativa L.	Codex Alimentarius Commission, 2018
Organoleptic qualities	The seeds have a pungent, bitter taste and smell; aromatic & peppery taste, persistent bitter taste on palate.	Margout et al., 2013; Bridgewater et al., 2021
Packing and storage	Should be stored away from other spices.	Dubey et al., 2016
aCASN = Chemical Abstracts Service Number; bCASN numbers preceded by 977 indicates this substance does not have a true CAS number, but one assigned by FDA as a convenience; cFEMA = Flavor and Extract Manufacturers' Association; dFEMA #2237 and 2342 – no reported uses of either substance at the time of original publication in GRAS 3 (Hall and Oser, 1965); eNeither this FEMA number nor CASN are recognized by FDA.		

Constituents of Nigella sativa seeds

Reports of the analysis of Nigella sativa seeds have consistently shown a wide variability in the presence and concentration of various constituents. For example, authors have reported a range of fixed oil, essential oil, protein, carbohydrate, minerals, et cetera.

Table5: Typical reports of composition of N. sativa seeds.

Constituents	Chemical composition	Reported Range (%)
Oil		
Fixed oil		22 – 38
Essential oil		0.4 – 2.5
Protein	Glutamic acid, arginine, aspartic acid, leucine, glycine, valine, lysine, threonine, phenylalanine, isoleucine, histidine, methionine	20.8 – 31.2
Carbohydrate	Glucose, rhamnose, xylose, arabinose	24.9 – 40
Minerals	Calcium, phosphorus, iron, potassium, sodium zinc, magnesium, manganese, copper, selenium	3.7 – 7
Saponins	α-Hederin (melathin), hederagenin (melanthigenin)	0.013
Alkaloids		0.01
Isoquinolines	Nigellimine, nigellicimine, nigellicimine N-oxide	
Pyrazoles	Nigellicine, nigellidine	
Other	Vitamin A, thiamin, riboflavin, pyridoxine, niacin, folacin, vitamin C	1 – 4
The fixed oil includes the unusual C20:2 eicosadienoic acid and the C20:0 saturated arachidic acid.		

Variations in macro-constituents are also reported from analyses of N. sativa seeds gathered from various parts of the world.

Table6: Nutritional composition of Nigella sativa seeds by country.

Country	Fat (%)	Protein (%)	Ash (%)	Total carbohydrate (%)	Fiber (%)
Tunisia and Iran	28.48 – 40.35	22.6 – 26.7	4.41 –4.86	32.7 – 40	NR
Tehran (Iran)	31.72	23.07	5.29	34.91	NR
Egypt	34.8	20.8	3.7	33.7	NR
Pakistan	31.6	22.80	4.20	NR	NR
Riyadh, Saudi Arabia	38.2	20.9	4.04	31.9	NR
Pakistan	21.67	24.05	4.34	39.04	5.5a
India, Syria and Turkey	39.1 – 42.5	19.9 – 24.1	4.2 – 4.8	NR	7.3 – 12.9a
Yemen	36.8 – 38.4	19.1 – 20.3	3.8 – 4.6	31.2 – 33.1	26.5 -36.8b 20.5 – 27.1c 6.5 – 8.9d
Iran	37.33	20.2	6.72	30.52	NR
West Malaysia	32.26	19.19	6.82	35.04	NR
aCrude fiber; bTotal dietary fiber; cInsoluble dietary fiber; dSoluble dietary fiber.					

There are also variations in the quantitative compositions of N. sativa essential and fixed oil constituents from various parts of the world. These differences may be attributed to several factors including, but not limited to, mistaken identity, adulteration, heredity, age of the plant, climatological environment, harvesting time, fertilization and irrigation regimens and other cultivation methods, distillation procedure (or solvents used) and isolation method [7].

Botnick also described a source of variance in volatile constituents of Nigella sativa seeds as the result of extraction of the seeds during various stages of maturation. The investigators described six stages of maturation – from stages one through three, the amount of essential oil is negligible – only when the flower is fertilized at Day 30, are volatiles produced in significant amounts, with γ-terpinene as the predominant volatile from Day 30 and peaking at Day 55 and subsiding by Day 60 to 75. The investigators describe Day 30 to 50 as the fourth stage, when black spots appear on the seed; at the fifth stage (Day 60), the seed is black in color and hardening and the sixth stage (Day 75) the seed is ripe – swollen and hard. During the fifth stage (Days 50 – 60) other major constituents start to peak (i.e., p-cymene, thymoquinone, thymohydroquinone, α-thujene and carvacrol). The decline in γ-terpinene may well be its conversion to p-cymene to carvacrol to thermohydroquinone and later to

thymoquinone. The minor players, α-pinene, sabine, β-pinene, etc., steadily increase from Day 30 to end stage maturation at Day 75 (Figure 1) (Botnick et al., 2012).

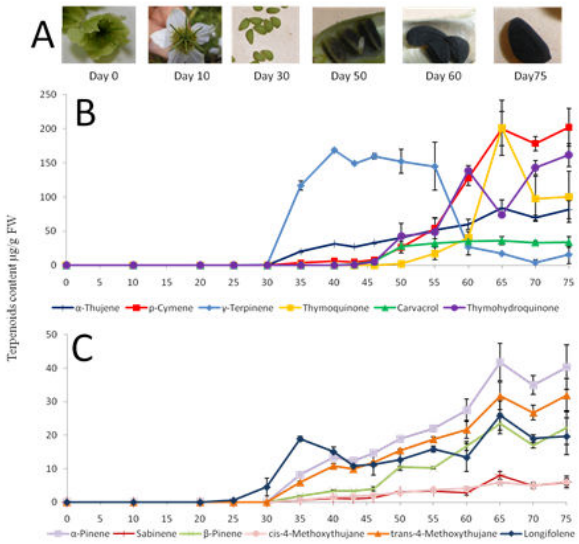

Figure1: Changes in volatile levels during N. sativa maturation. (A) Developmental stages of N. sativa seeds. (B) Major components. (C) Minor components.

Means and SE of three replicates of samples from Ein Harod source are shown.(Permission to reproduce this figure through the generosity of the authors.

Table7: Nigella sativa constituents of essential oil obtained from various world sources.

Substance	Source											
	India	Commercial sources in Austria	Iran	Egypt #1	Egypt #2	Egypt #3	Tunisia	Algeria	Syria	Jordan	Iran	Ethiopia
	Percent of totala											
	Majority of findings report constituent present at >6%											
α-Thujene	5.6	b	9.8	9.61	2.1	3.6	6.9	16.5	6.5	12.4	11.2	10.7
p-Cymene	31.4	7.07 – 15.53	37.3	30.5	18.8	24.1	60.5	36.5	73	36.8	64.6	66.5
Thymoquinone	37.6	27.8 – 57.0	13.7	30.2	63.3	56.4	3	14.7	6.2	18.4	9.7	9.6
	Majority of findings report constituent present at 1 – 5%											
Carvacrol	1.4	5.8 – 11.6	1.6	1.52	0.5	1.3	2.4	1.9		0.3		0.6
Limonene	1	0.29 – 3.30		3.23	2.4	1.7	1.4	-	-	0.2	-	-
α-Pinene	1.4	0.2 - 2.0	3.1	2.21	0.5	0.9	1.7	-	2.1	3.2	1	1.9

β-Pinene	1.7	0.1 – 1.80	3.4	2.5	1	1.4	2.4	0.2	-	3.8	1.3	-
iso-3-Thujanol				3.65	5.7	4.5						
	Majority of findings report constituent present at <1%											
trans-Anethole		0.25 – 4.28		0.08	0.2	0.1					Trace	0.2
p-Anisaldehyde		<0.01 – 0.07										
β-Bisabolene									0.1		Trace	
Borneol		<0.01 – 0.47										
Bornyl acetate	0.2	0.28 – 0.82	0.2					0.5	0.5		0.2	
iso-Bornyl acetate										0.2	0.3	
δ-Cadinene								0.1		0.2	0.1	
Camphene		<0.01 – 0.05						0.1	-	1	-	-
Camphor		0.05 – 0.10	0.6					0.1	Trace	Trace		0.2
β-Caryophyllene								0.1	0.1	0.2	Trace	0.1
Carvacryl acetate									0.4	Trace	0.3	
Carvenone												1.9
Carvone	Trace	0.13 – 1.05						0.1	Trace	0.1	0.1	
1,8-Cineole	0.1	0.02 – 0.19					0.1					
Citronellyl acetate			0.4									
Citronellyl butyrate								0.1				
α-Copaene								0.2			Trace	
Cuminal	Trace								Trace	Trace	0.1	

p-Cymen-8-ol	trace	0.25 – 0.78					0.2			6.2		
p-Cymen-7-yl acetate									0.1			
Davanone			0.1									
γ-Dehydro-ar-himachalene								0.1				
iso-Dihydrocarveol									0.7			0.4
trans-Dihydrocarvone											0.1	
α-p-Dimethyl styrene								0.2			Trace	Trace
Dodecanal								0.1				
α-Eudesmol	0.4											
β-Eudesmol	0.5											
10-epi-γ-Eudesmol	0.3											
Geranial									0.2			0.2
2-Heptanal		<0.01 – 0.04										
Himachalol										0.2		
α-Humulene								0.1	0.1	Trace		
trans-Isoeugenol									0.1			
Karahanaenone								0.7	0.2	0.5		
trans-Limonene oxide								Trace			0.3	Trace
Linalool		0.13 – 0.44		0.59	0.4	0.6						
Longicyclene								0.1	Trace			
Longifolene	2	1.26 – 8.0	6.4	1.07	2.1	1.3	0.9	3.1	1.7	2.4	0.4	0.9

iso-Longifolene								0.4				
iso-Longifolol								0.2	Trace			
α-Longipinene	0.5		2.1	0.32	0.4	0.2		1.1	0.5	0.7	0.2	0.2
β-Longipinene										0.1	Trace	Trace
p-Mentha-1,3,8-triene								0.4	Trace	0.2	Trace	Trace
6-Methyl-α-ionone								0.1				
Methyl linoleate								0.2	0.1	0.3	Trace	
γ-Muurolene								0.2				
β-Patchoulene								0.1				
β-Myrcene		<0.01 – 0.40		0.1	0.05	ND	0.1					
2-Pentadecanone								0.1				
Pimaradiene								0.1	Trace	Trace	Trace	
α-Phellandrene	0.1											
β-Phellandrene		<0.01 – 0.08										
Sabinene	0.8	0.08 – 1.20	2.2	1.03	0.4	0.6	0.9	7.5	-	1.7	0.2	-
trans-Sabinene hydrate	0.1	0.05 – 0.21							0.2	0.1	0.1	Trace
cis-Sabinene hydrate		0.06 – 0.16						0.1				
cis-Sabinene hydrate acetate		0.1							0.1	0.1	0.1	Trace
α-Terpinene	0.2	<0.01 – 0.12	0.8	0.57	0.2	0.08	1			0.8	Trace	

γ-Terpinene	0.2	0.02 – 1.10	2	11.8	0.4	0.5	3.5	1.9	0.4	3	0.9	0.5
α-Terpineol	Trace		2.2	0.34	0.76	0.6						
Terpinen-4-ol	1	1.98 – 6.59	1	0.2	0.4	0.5	2.1	0.6	0.3	0.7	0.3	0.3
Terpinolene		<0.01 – 0.05							0.6	Trace	0.9	0.5
α-Thujone		0.03 – 0.20						0.1				
Thuj-3-en-10-al								0.1				
cis-Thujopsene								0.1				
Tridecanal								0.2				
2-Tridecanone		0.11 – 0.38						0.1				
Thymohydroquinone	3.4						0.4	0.3	0.5	0.3	Trace	0.7
Thymol	0.2	0.11 – 0.25	0.4		0.6	0.4						
2-Undecanone		0.11 – 0.47						0.2	Trace			
	Singh et al., 2014	Burits and Bucar, 2000	Hajhashemi et al., 2004	Edris, 2010	Edris, 2010	Edris, 2010	Bourgou et al., 2010	Lawrence, 2008	Lawrence, 2008	Lawrence, 2008	Lawrence, 2008	Lawrence, 2008
aColumns will not total one hundred because the amount of "unidentified" or "unknown" was not included. bAny cell for which no value is present, indicates no value was reported by the author(s).												

These quantitative differences are readily demonstrated in a comparison of volatile oil constituents and fixed oil constituents from various sources.

Table8: Nigella sativa constituents of fixed oil obtained from various world sources.

Ethiopian	Indian	Moroccon	Moroccon	Balıkesir Area, Turkey	Banglade sh	Turkey				
		Method of extraction								
	Pet ether in Soxhlet	Pet ether in Soxhlet	Cold pressed	Cold pressed	Cold pressed	Pet ether in Soxhlet	Cold pressed	Hexane in Soxhlet	Microwav e-assisted	
	(g/100g)									
Polyunsat. FA	61.39	82.9	82.9±0.5	84						
Monounsat . FA	20.94									
Saturated FA	14.66	16.8	16.8±0.5	16						
trans FA		<0.01								
Fatty acid	**Character-ization**	**Predominant Fatty Acids**								
		(g/100g)								
Myristic acid	14:00	0.2	1	1±0.1	0.14 – 0.15	0.23	0.13	0.14	0.14	
Palmitic acid	16:00	11.36	10.83	13.1	13±0.1	11.64 – 11.98	13.1	12.01	11.85	11.84
Palmitoleic	16:01	0.2±0.1	0.18 – 0.2	0.28	0.25	0.23	0.24			
Heptadeca noic acid	17:00	0.061 – 0.067	0.06	0.07	0.07					
Heptadese noic acid	17:01	0.05 – 0.056	0.03	0.04	0.04					
Stearic acid	18:00	2.81	2.47	2.3	2.3±0.1	3.32 – 3.35	2.47	2.77	2.95	2.81
Oleic acid	18:01	17.63	19.09	23.8	23.8±0.1	24.3 – 24.64	21.8	23.95	24.13	23.85
cis-11-Octadecen oic acid	18:01	1.18								
Linoleic acid	18:02	61.25	50.24	58.5	58.5±0.1	55.57 – 56.37	57	57.49	57.18	57.52
Linolenic	18:03	0.4	0.4±0.1	0.22 – 0.26	0.46	0.25	0.23	0.27		
Arachidic acid (Eicosanoi c acid)	20:00	0.19	0.5±0.1	0.2 – 0.26	0.18	0.15	0.16	0.16		
cis-11-Eicosenoic acid	20:01	3.04	2.25	0.25 – 0.36	0.39	0.27	0.29	0.31		
Eicosadien oic acid	20:02	2.55 – 2.58	2.91	2.33	2.45	2.49				
Behenic acid	22:00	0.02 – 0.039	ND							
Docosenoi c acid	22:01		0.033 – 0.048							
Lignoceric acid	24:00:00							0.31	0.28	0.26
		Thilaka-rathna *et al.*, 2018	Thilaka-rathna *et al.*, 2018	Gharby *et al.*, 2015	Asdadi *et al.*, 2014	Argon and Gökyer, 2016	Kabir *et al.* 2019	Kiralan *et al.*, 2014		

Specifications

No specifications or definitions for standards of identity for spices have been established in accordance with Section 401 of the Food Drug and Cosmetic Act, although advisory standards have been promulgated. These advisory standards were updated in consideration of promulgation of 21 CFR § 182 (GRAS spices and other natural flavorings) and 21 CFR § 101.22 (labeling) and in consultation with the American Spice Trade Association, the following policy and definitions have been published.

Table9: US FDA Compliance Policy Guideline (CPG) for Section 525.750 and Definition[a]

POLICY:	In the absence of definitions and standards of identity for spices, the following descriptions provide guidance concerning acceptable names for use in labeling spices and foods in which they are used. Only the commonly used spices are included; specific questions about other substances which may be considered as spices within the general definition may be referred to the Food and Drug Administration.
DEFINITIONS:	SPICES - General Definition - Aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition. They are true to name and from them no portion of any volatile oil or other flavoring principle has been removed.
aIn this CPG, several spices ae defined in terms of genus and species, appearance and flavor/odor. Nigella sativa is not defined.	

In 2013, FDA released its "Draft Risk Profile: Pathogens and Filth in Spices" and disclosed that samples of Nigella were reported to have contained Salmonella spp. and Salmonella serotype Weltevreden. Whether or not in response to the FDA warning, some suppliers have indicated to their customers in their N. sativa product specifications that although the product is not sterile, it has been "heat treated…to reduce the microbiological levels within the product". Further, "…if our products are used for food use, goods must be cooked thoroughly before use/consumption".

According to Malhotra, the Indian Agmark grade specifications for Nigella sativa L. seeds have been promulgated under the Indian Prevention of Food Adulteration Indian Act (PFA standards).

The aforementioned author indicates the Nigella seed has yet to be included in specification lists for the American Spice Trade Association, the European Spice Association or International Organization for Standardization (ISO).

Table10: Indian Agmark specifications for Nigella sativa seeds.

Parameter	Boundaries
Seed moisture	Not more than 11% by weight
Total ash	Not more than 6% by weight
Ash insoluble in acid	Not more than 1% by weight
Organic extraneous matter(s)	Not more than 3% by weight
Inorganic extraneous matters	Not more than 2% by weight
Volatile oil	Not less than 1% (v/w)
Ether extract (crude oil)	Not less than 35% (v/w)
Alcoholic acidity as oleic acid	Not more than 7% (v/w)

The quality of whole N. sativa seed is largely assessed on appearance – the seeds should be matt-black, with an oily white interior and roughly triangulate, 1.5 – 3 mm long with a uniform size, shape and texture. Malhotra adds that N. sativa seed is subject to adulteration by onion seed (fresh or spent) because of their morphological similarity to N. sativa seeds.

Economic applications

Uses as a food ingredient

N. sativa seeds are used in the Middle East and India as a spice and condiment and sporadically in Europe as a substitute for pepper or other "biting" spices. The seeds have a strong aroma and because they have a pungent–bitter taste, they are often eaten in combination with honey. The seeds are widely used as a minor ingredient in food preparation including pickles, naan (a type of Indian flat bread), and other bakery products to give a characteristic flavor. In the Middle Eastern countries, N. sativa seeds are used in bread dough and are a vital component of Armenian choereg rolls. N. sativa seeds (sometimes in a roasted format) are widely used to flavor the curries, dhal (a lentil stew), chutney and several other culinary preparations including mildly braised lamb dishes such as korma (a stew with meat or vegetables braised with yogurt or cream and spices to produce a thick sauce) and in Mediterranean cheeses as a preservative. The seeds are also added to coffee, tea and bread, canned foods, wine and vinegar. N. sativa seeds, seed meal, and oil are also used as adjuncts to give characteristic flavor and because the essential oil contains some strong antioxidants (e.g., thymoquinone), it also serves as a food preservative.

N. sativa seeds are also used as an ingredient in a mixture of spices (garam masala) and is a crucial part as one of the five spices in panch phoran, a combination consisting of N. sativa seed, fenugreek seed, black mustard seed, cumin seed, and fennel seed. The panch phoran originated from Eastern India and is used in the area of its origin, as well as Nepal and Bangladesh.

Non-food uses

Nigella sativa essential oil has also been used as an insect repellant. N. sativa seeds are scattered between folds of linen and wool to stop insect attack. Essential oil extracts are used in range of beauty products like hair oil, body oil, soaps and shampoos. A potent insecticidal effect has also been reported from N. sativa essential oil. The saponin, α-hederin (also known as melathin), from the seeds has been reportedly used as a fish poison. Karimi, in their review of the uses of Nigella sativa and, its essential oil constituent the powerful antioxidant, thymoquinone, have suggested that the addition to food has prevented the toxic effects of several substances.

Table11: Nigella sativa and thymoquinone preventing or ameliorating the effects of food-borne toxins.

Metals	Antibiotics	Food processing toxicants	Mycotoxins
Arsenic	Oxytetracycline	Diethylnitrosamine	Zearalenone
Cadmium	Gentamicin	Acrylamide	Ochratoxin A
Mercury	Pesticides	Benzo[a]pyrene	Verrucarin
Aluminum	Acetamiprid	Bisphenol A	AflatoxinB1
Iron	Chlorpyrifos	Food additive toxins	
Chromium	Chlorpyrifos	Tert-Butylhydroquinone	
	Diazinon	Carrageenan	
	Propoxur		

N. sativa and its extracts have found considerable use as folk medicine in the Middle East and it is said the Islamic prophet Muhammad, described N. sativa seeds as being able to "heal every disease except death". A partial list of medical uses is provided.

Table12: Medical usesa of Nigella sativa seed and extracts thereof (Ahmad et al., 2013).

	General use as a(n)		For treatment of
Diuretic	Immunomodulator	Antioxidant	Diabetes
Analgesic	Antimicrobial	Anti-oxytocic	Bronchitis
Anthelminthic	Anti-inflammatory	Antifungal	Diarrhea
Spasmolytic	Bronchodilator	Anticonvulsant	Skin disorders
Gastro-protective	Hepato-protective	Contraceptive	Hypertension
Testicular protective	Neuro-protective	Immunostimulant	Cancer
Renal protectant	Liver tonic	Cardio-protective	Asthma
Emmenagogue	Increase milk production		Rheumatism

Violative uses

The vast majority of publications focus on the biomedical application of N. sativa L. seeds and their extracts, although they are not an approved drug and such claims are not allowed by FDA. In a warning letter to one company, FDA made reference to several drug claims made by the company for their "Black Seed Oil" including, but not limited to killing MRSA (methicillin-resistant Staphylococcus aureus), stimulating regeneration of the dying beta cells within the diabetic's pancreas, cold and flu prevention, elimination of intestinal parasites and, possessing anticonvulsant activity and effects on hypertension, asthma, colon cancer, opiate addiction, skin disease, et cetera. FDA also accused the company of health fraud.

A second company was found in violation of Current Good Manufacturing Practice (CGMP) regulations for Manufacturing, Packaging, Labeling or Holding Operations for Dietary Supplements (21 CFR Part 111). FDA noted that "These violations cause your dietary supplement products, including but not limited to Diabalance Herbal Blood Sugar Balance Tablets, Quick Slim with pure Hoodia Gordonii Capsules, BHealthy Black Seed Bitter Melon 90 Veggie Capsules, Black Seed w/ Olive Leaf/ Garlic Capsules, Black Seed Honey Booster, Sweet Excitement Her Honey, Revive Honey Herbal Elixir, Flax Seed Oil, Black Seed Oil, and Black Cumin Seed Oil, to be adulterated within the meaning of section 402(g)(1) of the Federal Food, Drug, and Cosmetic Act (the Act) [21 U.S.C. § 342(g)(1)], in that they have been prepared, packed, or held under conditions that do not meet the cGMP regulations for dietary supplements found under 21 CFR Part 111". The FDA further noted "Additionally, we reviewed your website […] in April 2017 and determined that you take orders there for the products: Diabalance Herbal Blood

Sugar Balance Tablets, Quick Slim with pure Hoodia Gordonii Capsules, BHealthy Black Seed Bitter Melon 90 Veggie Capsules, and Black Seed w/ Olive Leaf/Garlic Capsules. The claims on your website and on the product labels that we collected during the inspection establish that these products are drugs under section 201(g)(1)(B) of the Act [21 U.S.C. § 321(g)(1)(B)], because they are intended for use in the cure, mitigation, treatment, or prevention of disease. … [i]ntroducing or delivering these products for introduction into interstate commerce for such uses violates the Act. Further, we have reviewed the labels used on several of your products and have identified significant violations causing the products to be misbranded within the meaning of section 403 of the Act (21 U.S.C. § 343).

Companies making similar drug-type claims also received warning letters, including one company alleging "anti-diabetic capacity of BSO" (black seed oil), treatment of asthma, eczema, inflammation, influenza and diseases). The company cited many published articles in support of its claims; however, FDA indicated that these products were drugs because they are intended for use in the cure, mitigation, treatment or prevention of disease and introducing or delivering these products into interstate commerce violates the Act (i.e., the Federal Food, Drug, and Cosmetic Act).

Regulatory history

Nigella sativa seed has been approved for use in food by FDA and incorporated into 21 CFR § 182.20 in 1977 (42 FR 14640 March 15, 1977) as one of many substances used prior to January 1, 1958, and therefore GRAS on the basis of common use in food. However, this GRAS statis is valid only when N. sativa is used for the purposes indicated and, in a manner consistent with good manufacturing practice. That is, N. sativa is permitted only as a flavoring agent or adjuvant in the US, not as a preservative as is the practice in some other countries. Further stipulations for use in food and maintenance of GRAS status for the substance are provided in Table14.

Table13: United States regulatory status of Nigella sativa.

Authoritative body	Citation/Comments	Food category	Permitted functionality	Use limits
FDA	a,b21 CFR § 182.10 Spices and other natural seasonings and flavorings. (as Caraway, black (black cumin), Nigella sativa L.	No restrictions	c(12) Flavoring agents and adjuvants	cGMP
FDA	a21 CFR § 582.10 Spices and other natural seasonings and flavorings. (as Caraway, black (black cumin), Nigella sativa L.	Animal feed; not res tricted to a specific speciesb	(12) Flavoring agents and adjuvants	cGMP
AAFCO	Spices and other natural seasonings and flavorings (AAFCO, 2021)	Animal feed; not restricted to a specif ic species	Seasonings and flavorings	cGMP
FEMA	GRAS No. 2237 Caraway, black (Nigella sativa L.) (Hall and Oser, 1965)	No specific food categories approved	Flavor ingredient	No reported uses
FEMA	GRAS No. 2342 Cumin, black (Nigella sativa L.) (Hall and Oser, 1965)	No specific food categories approved	Flavor ingredient	No reported uses

AAFCO = Association of American Feed Control Officials; CFR = Code of Federal Regulations; cGMP = Good Manufacturing Practice; CoE = Council of Europe; FEMA = Flavor and Extract Manufacturers' Association; FDA = US Food and Drug Administration; GRAS = Generally Recognized As Safe.

aInterestingly, while most substances listed in 21 CFR § 182.10 or 582.10 (whole spices) were carried over into 21 CFR § 182.20 or 582.20 (essential oils, oleoresins and natural extractives), Nigella sativa was not.

bAlthough several plants in this regulation concerning spices and seasonings specifically designate a part of the plant (e.g., ambrette seed, angelica seed, celery seed), no distinction is made for the seed of the Nigella sativa L plant. However, in the literature reviewed for this publication, no reference was found indicating any other part of the plant was consumed, other than the seed.

cPermitted functionalities are provided in 21 CFR § 170.3(o).

Table14: Nigella seed: stipulations for use in food and preservation of GRAS status.

21 CFR § 182.1(b) For the purposes of this section, good manufacturing practice shall be defined to include the following restrictions:
(1) The quantity of a substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional, or other technical effect in food; and
(2) The quantity of a substance that becomes a component of food as a result of its use in the manufacturing, processing, or packaging of food, and which is not intended to accomplish any physical or other technical effect in the food itself, shall be reduced to the extent reasonably possible.
(3) The substance is of appropriate food grade and is prepared and handled as a food ingredient. Upon request the Commissioner will offer an opinion, based on specifications and intended use, as to whether or not a particular grade or lot of the substance is of suitable purity for use in food and would generally be regarded as safe for the purpose intended, by experts qualified to evaluate its safety.

Importantly, the GRAS status of the substance and listing in this section of the CFR is temporary and that "when the status of a substance has been reevaluated, it will be deleted from this part [21 CFR 182.10] and will be issued as a new regulation … as 'affirmed as GRAS'" under 21 CFR § 184 or other appropriate category (which includes 21 CFR § 189 "prohibited from use in food").

As noted in the table above, when substances were nominated for GRAS status in the 1970's, many of the whole spices and seasonings (i.e., whole plants, plant parts or seeds) or extracts of these spices and seasonings were included in 21 CFR § 182.20, although Nigella sativa L. was not. No reason could be found for this exclusion, other than the presumption that the prevailing common knowledge at the time was that only the seed had ever been consumed and was never extracted. However, a check of the internet reveals N. sativa oil is readily available from online vendors, although this does not necessarily mean that all the products for sale in e-commerce are approved or safe [8].

FDA has declined to file at least two New Dietary Ingredient Notifications (NDIN) for N. sativa L. (or otherwise described as "black seed" or "blessed seed") for one company on the basis that the notification was incomplete, and that the product may be adulterated and does not provide reasonable assurance that the ingredient did not present a significant or unreasonable risk of injury (the standard of safety for dietary supplement ingredients).

Consumption

No quantitative estimates of consumption in the United States were possible, as such values are based on importation and/or survey data, neither of which were available. BIOLOGICAL DATA

Toxicological studies

Acute (single dose) toxicity studies

Essential oil single dose studies

Amina (2016), using Nigella sativa essential oil (source and method of obtaining the oil was not disclosed), with a polyphenol equivalent to gallic acid of 10.674 µg, (orally?) dosed Wistar rats (5 animals per group) of both sexes and whose body weights ranged from 180 to 300 grams with 0.2, 0.4, 0.6, 0.8 and 1 mg essential oil/kg. This same investigator also dosed 95 albino mice (NMRI strain) of both sexes, whose weight ranged from 17 – 23 grams; presumably the same doses were administered to the mice as were the rats. The author reported no deaths and an LD50>1 mg/kg bw for both species as that was the highest dose tested.

El-Hadiyah obtained the essential oil (called "volatile" oil by the authors) from Nigella sativa seeds (of Ethiopian origin) by first crushing the seeds, steam distillation of the seeds and the oil separated by the use of diethyl ether, the latter of which was eliminated by vacuum distillation. The yield of the volatile oil was 6.2 ml per 1 kg crushed seeds. The oil was not further characterized. Male Swiss albino mice (20 – 25 grams) were used for the intraperitoneal LD50 determination; a preliminary range-finding study was first used to approximate the dose to be administered and finally, the five dose levels for the LD50 were administered to five animals per group in a dose-responsive manner, the animals were observed for 24 hours post-dose and the LD50 was calculated by the method of Litchfield and Wilcoxon (1949). The intraperitoneal LD50 for male Swiss mice for the volatile oil was LD50=1853 mg/kg bw (95% CL=1634-2106).

Fixed oil single dose studies

Zaoui et al. (2002) obtained fixed oil from Moroccan-sourced N. sativa seeds by extracting the mechanically powdered seeds with hexane and removal of the solvent under vacuum. The authors note identification of the oil was carried out according to the procedure described in the British Pharmacopoeia. The oil was found to contain the fatty acids: myristic, palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids; triterpenes and saponosides. The authors then conducted a single, oral dose (acute) toxicity study with the hexane-extracted fixed oil, using IOPS OFA mice, 8 – 10 weeks of age and weighing 20 – 22 grams each. The oil was administered by gavage to each of 10 groups of mice (5 males and 5 females) of 10, 15, 20, 25, 30, 40 or 50 ml/kg bw of the fixed oil. The animals were observed for gross effects and mortality during the 15 days following the oil administration. Post-mortem examinations were carried out on the dead animals. The animals exhibited immediate agitation and behavioral activity with temporary writhing, followed by a quiet period and sedation. Diarrhea was observed and the animals died within 12 hours. Dead animals exhibited congested lungs and hearts stopped in diastole. Surviving animals recovered quickly within a period of 4 – 8 days. The authors reported an oral LD50 of 28.8+1 (mean and standard deviation, respectively) and (confidence interval (CI): 26.2-31.6) ml/kg bw.

Zaoui conducted a single, intraperitoneally injected dose of hexane extracted N. sativa oil into IOPS OFA mice, 8 – 10 weeks of age and weighing 20 – 22 grams. Each group of 10 males and 10 females received 0.25, 0.5, 1, 2, 3, or 6 ml/kg bw prepared in

gum acacia (5%). The animals were observed for gross effects and mortality during the 15 days following the oil administration. Postmortem examinations were carried out on the dead animals. Post-administration observations were the same as following oral dosing. The authors reported an intraperitoneal LD50 of 2.06+0.1(CI:1.86-2.26) ml/kg bw.

El-Hadiyah et al. (2003) reported Nigella sativa fixed oil was extracted from the seeds (of Ethiopian origin) using hexane. The yield was 240 ml of oil per 1 kg crushed N. sativa seeds. The accompanying volatile oil was removed using steam-distillation. The remaining fixed oil was then purified using silica gel column chromatography. The yield of purified fixed oil was 152 ml per 240 ml total oil, i.e. 152 ml fixed oil per 1 kg crushed N. sativa seeds. The oil was not further characterized. Male Swiss albino mice (20 – 25 grams) were used for the intraperitoneal LD50 determination; a preliminary range-finding study was first used to approximate the dose to be administered and finally, the five dose levels for the LD50 were administered to five animals per group in a dose-responsive manner, the animals were observed for 24 hours post-dose and the LD50 was calculated by the method of Litchfield and Wilcoxon The intraperitoneal LD50 for male Swiss mice for the fixed oil was LD50=3371 mg/kg bw (95% CI=2676-5109).

Whole seed extracts

El-Hadiyah et al. (2003) prepared an aqueous extract (AE) of N. sativa seeds of Ethiopian origin. The seeds were finely crushed, washed with n-hexane and macerated in water for 48 hours with occasional shaking. After filtration, the solvent (water) was evaporated under vacuum. The yield of the AE was 116 g per 1 kg of crushed N. sativa seeds. The extract was not further characterized. Male Swiss albino mice (20 – 25 grams) were used for the intraperitoneal LD50 determination; a preliminary range-finding study was first used to approximate the dose to be administered and finally, the five dose levels for the LD50 were administered to five animals per group in a dose-responsive manner, the animals were observed for 24 hours post-dose and the LD50 was calculated by the method of Litchfield and Wilcoxon (1949). The intraperitoneal LD50 for male Swiss mice for the aqueous extract was 3020 mg/kg bw (95% CL=2530-3610-3610).

Vahdati-Mashhadian produced aqueous, methanol and chloroform extracts of Nigella sativa seeds for performance of an oral LD50 in mice. The seeds were obtained locally (Mashhad, Iran). Aqueous and methanol extractions were performed by reflux extraction, wherein 150 grams of powdered N. sativa was extracted with 500 ml of water or methanol for 12 h at 95°C and 70°C, respectively. The extracts were filtered using filter paper and dried for 24 hours at 50°C. For the chloroform extraction, 1500 grams of ground N. sativa seed was incubated at 25°C in chloroform for four days and agitated twice per day. The solution was filtered and dried the same as the water and methanol extracts. The extracts were not further characterized. The aqueous and methanol extracts were dissolved in distilled water and the chloroform extract in sesame oil for administration. Animals of each sex (5 – 6 weeks of age, weighing 20 – 25 grams) were used. The animals were fasted six hours prior to dosing and under close observation 72 hours post-dose. To determine the LD50 values of the extracts, the extracts (aqueous, methanol and chloroform) were administered once, per os, in four different dose groups of 6, 9, 14 and 21 g/kg bw. Mortality and body weight changes were recorded at 3 and 7 Days post-dose. No mortalities were reported with any of the extracts. The methanol- and chloroform- extract groups at the 21 g/kg bw dose level were reported to have a significant decrease in body weight compared to controls at Days 5, 6 and 7 (Vahdati-Mashhadian et al., 2005.

Extracts of seed hulls

Michel, investigated the effects of extracted seed waste (N. sativa hulls). The waste was obtained from a local milling operation in Cairo, Egypt and subjected to a series of extractions.

The hexane extraction (HE) was obtained by re luxing 100 g of seed waste with n-hexane at 40 – 60°C; the ethanol extraction (EE) was an extraction of the biomass from the hexane extraction; both extracts were evaporated to dryness. Another tranche of seed waste was subject to aqueous extraction (AE) by being boiled with distilled water for four hours then iltered and concentrated.

The investigators reported the LD50 for all extracts to be 5000 mg/kg bw as determined by the Miller and Tainter method but gave no additional details on the experimental design used to derive the LD50 [9].

Table15: Summary of acute (single dose) toxicity studies.

Substance	Source & Method	Route &Dose	pecies & Sex	bservation Period	LD50	eference
Essential oil, not characterized	Not disclosed	Oral(?); 0.2, 0.4, 0.6, 0.8, 1.0 mg/kg	Wistar rats, M & F	Not disclosed	No mortalities, LD50 >1.0 mg/kg	Amina, 2016
Essential oil, not characterized	Not disclosed	Oral(?); 0.2, 0.4, 0.6, 0.8, 1.0 mg/kg	Albino mice (NMRI),	Not disclosed	No mortalities, LD50 >1.0 mg/kg	Amina, 2016
			M & F			
Essential oil, not characterized	Ethiopian seeds, steam distillation & diethyl ether extraction	Intraperitoneal; doses not disclosed	Swiss albino mice, M	24 hr	LD50=1853 mg/kg bw	El-Hadiyah et al., 2003
Aqueous extract, no t characterized	Ethiopian seeds were crushed,	Intraperitoneal; doses not disclosed	Swiss albino mice, M	24 hr	LD50=3020 mg/kg bw	El-Hadiyah et al., 2003

	washed with hexane and macerated in water					
Aqueous extract, not characterized	Iranian seeds, powdered seeds reflux extracted with water for 12 hours at 95°C, then extract filtered & dried; reconstituted with water	Oral; 6, 9, 14 & 21 g/kg	Mice	72 hr	No mortalities, LD50>21g/kg	Vahdati-Mashhadian et al., 2005
Methanol extract, not characterized	Iranian seeds, powdered seeds reflux extracted with water for 12 hours at 70°C, then extract filtered & dried; reconstituted with water	Oral; 6, 9, 14 & 21 g/kg	Mice	72 hr; BW loss at highest dose	No mortalities, LD50>21g/kg	Vahdati-Mashhadian et al., 2005
Chloroform extract, not characterized	Iranian seeds, powdered seeds incubated in chloroform for four days at 25°C, then extract filtered & dried; delivered in sesame oil	Oral; 6, 9, 14 & 21 g/kg	Mice	72 hr; BW loss at highest dose	No mortalities, LD50>21g/kg	Vahdati-Mashhadian et al., 2005
Fixed oil, not characterized	Ethiopian seeds were crushed & extracted with hexane & steam distillation	Intraperitoneal; doses not disclosed	Swiss albino mice, M	24 hr	LD50=3371 mg/kg bw	El-Hadiyah et al., 2003
Fixed oil, fatty acids identified; not otherwise characterized	Morroccan seeds were ground to a powder & extracted with hexane	Oral; 10, 15, 20, 25, 30, 40 or 50 ml/kg bw	Mice, M&F	15 days	LD50=28.8 ml/kg bw	Zaoui et al., 2002
Fixed oil, fatty acids identified; not characterized	Morroccan seeds were ground to a powder & extracted with hexane	Intraperitoneal; 0.25, 0.5, 1, 2, 3, or 6 ml/kg bw	Mice, M&F	15 days	LD50=2.06 ml/kg bw	Zaoui et al., 2002
Aqueous extract of seed waste, not characterized except amino acids	Egyptian seed waste (hulls) was ground to a powder and boiled in water X 4 hours	Oral	Albino mice, male	Not reported	LD50>5000 mg/kg bw	Michel et al., 2011
Hexane extract of seed waste, not characterized except amino acids	Egyptian seed waste (hulls) were ground to a powder and extracted with hexane	Oral	Albino mice, male	Not reported	LD50>5000 mg/kg bw	Michel et al., 2011
Hexane/EtOH extract of seed waste, not characterized except amino acids	Egyptian seed waste (hulls) was ground to a powder, extracted with hexane, followed by an extraction by ethanol (70%)	Oral	Albino mice, male	Not reported	LD50>5000 mg/kg bw	Michel et al., 2011

Irritation

Evaluated the irritation and sensitization potential of an N. sativa oil in an herbal analgesic cream. N. sativa seeds were obtained in a local market (Karachi, Pakistan) and authenticated by a botanist for confirmation of identity. The seeds were ground and extracted with n-hexane, which was removed by distillation and the residue was dried. Five grams of the oil was extracted with methanol, run through a silica column with petroleum ether and standardized against purified thymoquinone (see original paper for a complete description of the purification procedure of the N. sativa oil). The N. sativa oil was added to a cream containing borax, was, propylene glycol, liquid paraffin, stearic acid, cetyl alcohol, propyl paraben, methyl paraben, water and N. sativa oil, the latter of which was 5% of the mixture. An acute dermal test of the cream was conducted with rabbits according to the OECD Guideline 404. The investigators applied 0.5g cream or an equal amount of cream sans N. sativa to six square centimeters and held in contact with the skin by a non-occluding bandage. After four hours, the test

and control material were removed. The sites were examined for erythema and edema at 1, 24 and 72 hours. Negligible erythema was seen in one animal each in test and control at 1 hour, the sites cleared at 24 hours and the irritation index score was 0.4, which according to the Draize method, classifies this cream with N. sativa as a non-irritant.

Repeated dose and subchronic toxicity studies (Table 16)

Studies up to seven days in length

In a five-day gavage study in rats, Al-Ghamdi examined the effect of an aqueous suspension of N. sativa seeds on male albino rats. Dry N. sativa seeds were purchased from a local market in Dammam, Saudi Arabia. 12.5 Grams of the seeds were ground and added to 100 ml distilled water a few minutes prior to dosing the animals, the suspensions were not further characterized. Adult albino Wistar male rats (age >24 months, weighing 250 – 300 grams) were divided into groups of six each. Group #1 (the control) received water only by gavage, Groups #2 and #3 received N. sativa suspensions by gavage of 250 and 500 mg/kg for five days and 2 µL/kg olive oil on Days 4 and 5 (the purpose for administration of the olive oil was to act as a vehicle control for other groups receiving carbon tetrachloride). The investigator measured serum aspartate transaminase (AST), L-alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) and, in addition, took samples of liver tissue for histopathological examination. On Day 6, the animals were anesthetized with ethyl ether, blood and tissues were collected. Following termination, the analysis of serum AST, ALT and LDH for 250 or 500 mg N. sativa/kg bw/day did not significantly differ from vehicle control value, nor was there any difference in histopathological observations from N. sativa treated vs the vehicle control value.

Studies greater than seven days and up to five weeks in length

In a 14-day gavage study in rats, El Daly (1994) sought to examine the effect of a water extract of N. sativa seeds on carbohydrates and the liver of male rats. The investigator obtained N. sativa seeds from the local market in Aswan, Egypt. The seeds were washed to remove sand and debris, the seeds were dried, and 300 grams of the dried seeds were boiled in distilled water (1200 ml) for 90 minutes. The decoction was filtered through muslin, resulting in a 384 ml filtrate; the filtrate was not further characterized. Adult male albino rats (150 – 170 grams were selected for study and divided into two groups of nine each (Groups 1 and 2) and two groups of 10 each (Groups 3 and 4). The aqueous extract of the N. sativa seed was administered by gavage (10 mL/kg) to Group 1 for seven consecutive days and an equal amount was administered to Group 2 animals for 14 consecutive days. Group 3 received an equal amount of normal saline for 14 consecutive days and Group 4 served as untreated controls. All oral dosing was performed under light ether anesthesia. At the end of the experimental period (7 days for Group 1 and 14 days for Groups 2, 3 and 4), the animals were killed by cervical dislocation, blood was collected, and livers harvested. Following termination, in a comparison of the saline and untreated controls, the blood glucose levels were significantly decreased in N. sativa treated Groups 1 and 2 and, the insulin levels were increased compared to controls, as were the alanine transaminase and γ-glutamyl transpeptidase values. There was no difference between treated vs control animals for aspartate aminotransferase or alkaline phosphatase values. Histopathological examination of the livers showed no evidence of degenerative changes in hepatocytes in any control or treated animals. The investigator noted some dilatation of central veins and surrounding sinusoids in most all gavaged animals and attributed the effect to the use of ether anesthesia, but the dilatation was somewhat enhanced in N. sativa treated animals. Some mononuclear cell infiltrate was noted in N. sativa treated animals, but less so in saline-treated and untreated animals. The investigator hypothesized that some changes may have occurred in the liver at a molecular level and could not rule out possible enzyme induction by N. sativa. The investigator concluded that "the repeated use of this medicinal plant as therapeutic agent should not be encouraged."

For a 14-day repeated dose study in rats, Vahdati-Mashhadian produced aqueous, methanol and chloroform extracts of Nigella sativa seeds for performance of an oral, repeated administration toxicity study in male mice (strain not identified). The seeds were obtained locally (Mashhad, Iran). Aqueous and methanol extractions were performed by reflux extraction, wherein 150 grams of powdered N. sativa was extracted with 500 ml of water or methanol for 12 h at 95°C and 70°C, respectively. The extracts were filtered using filter paper and dried for 24 hours at 50°C. For the chloroform extraction, 1500 grams of ground N. sativa seed was incubated at 25°C in chloroform for four days and agitated twice per day. The solution was filtered and dried the same as the water and methanol extracts. The extracts were not further characterized. For administration to the animals, the aqueous and methanol extracts were dissolved in distilled water and the chloroform extract in sesame oil. The male mice were administered a single daily dose of 6 grams/kg bw of the aqueous, methanol or chloroform extracts for 14 consecutive days; in addition, there was an untreated group (receiving nothing, nor were they sham dosed), Control #1 received distilled water (a control for the aqueous and methanol extracts) and Control #2 received sesame oil (a control for the chloroform extract, whose results are not reported here). The authors, indicated there was no significant reduction in animal body weight over the course of the study. The administration of the aqueous extract resulted in decreased serum levels of alkaline phosphatase (ALP), but no change in aspartate aminotransferase (SGOT) or serum alanine aminotransferase (SGPT). The methanol extract group did not show any changes in hepatic enzymes. The chloroform extract did not show a significant change in ALP, but this extract did cause a significant decrease in SGOT and SGPT. Hepatic histopathologic changes were seen in all test animals; however, the authors reported that variable degrees of cellular inflammation and dilation of central and peripheral veins were observed in normal and Controls #1 and #2.

Tennekoon administered a water extract of N. sativa seeds to rats by gavage for 14 days then measured liver enzymes and examined the livers histopathologically. N. sativa seeds were obtained from a local (Colombo, Sri Lanka) Ayurvedic drug store; the seeds were washed, air-dried and 250 grams were boiled in distilled water (1000 mL) for 90 minutes and filtered through muslin. The final volume of the filtrate was 320 mL; the extract

was not further characterized. Five-month-old male Sprague-Dawley rats were divided into a control group of 10 rats and a treatment group of eight rats, the latter of which received a daily dose of 10 mL/kg of N. sativa extract for 14 days. The control group received an equal amount of water for 30 days. At the end of the experimental period, the animals were killed, and blood obtained via cardiac puncture. The serum obtained from the blood draw was analyzed for γ-glutamyl transferase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. The authors reported that serum γ-glutamyl transferase for the N. sativa-treated animals was significantly elevated over control values, although alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were not. Histopathological examination of the livers did not show evidence of degenerative changes in the treated animals.

In a 30-day study repeated dose study, Zaghlol using three groups of seven adult male albino rats each, consisting of a control (Group 1) and Groups 2 and 3, with each animal in the respective group having been administered gradually increasing doses of 15 and 25 ml of otherwise undefined N. sativa oil/kg bw for one month, whereupon the animals were sacrificed and, samples of liver and kidney were taken for histological examination. The authors reported that in Group 2, the kidney exhibited epithelial shedding and necrosis of some cells in the proximal and distal convoluted tubules, but no effect on the glomeruli. In Group 3, glomerular injury was reported in the form of degeneration of the glomerular tuft, an ill-defined basement membrane and destruction of endothelial cells, in addition of tubular necrosis. In Group 2, there was a minimal effect on the liver in the form of perivascular cellular infiltration, although in Group 3 there was markedly vacuolated foamy cytoplasm in hepatocytes, with dilated sinusoids and perivascular cellular infiltration. The authors concluded “large doses of N. sativa oil have toxic effects on the histological structure of the kidney and to a lesser degree on the liver”.

For a 35-day feeding study with rats, Dollah obtained N. sativa seeds from India at a local market in Malaysia. Following a visual authentication, the seeds were cleaned with tap water, rinsed twice with distilled water and dried overnight at 40°C. The seeds were ground to a powder and mixed with rat chow pellet powder and water into different doses of 0.01, 0.1 and 1 g/kg bw of the rats. Twenty-four male Sprague Dawley rats with 300 – 350 g body wight were assigned into four treatment groups of 0.0 (control), 0.01, 0.1 and 1 g ground N. sativa ground seeds/kg bw. The animals were allowed unlimited access to the feed for five consecutive weeks. According to the authors, “the dosages were chosen based on human N. sativa consumption which is equal to 2 g/day and considering conversion rate to rats, 0.1 g/kg was selected as the normal dose.” At the conclusion of the five weeks of treatment, the authors indicated there was a statistically significant difference in all groups compared to body weights at the study initiation, but no comparison was made between treatment groups and control values. Serum creatinine levels were decreased in high dose animals (1.0 g/kg bw/day) compared to control, low- and mid-dose animals. Serum urea levels were increased in low-dose animals compared to mid-dose animals, although no statistically significant differences in control value compared to low-, mid- or high-dose values. Histopathological examination of the kidneys did not reveal any changes in any groups.

In 28-day follow-up study in male Sprague-Dawley rats, Dollah using the same N. sativa extract, and protocol, with the exception that the animals were treated for only four weeks, instead of five in the kidney study. In this study, the objective was to assess liver toxicity by examination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and histopathological examination. At the end of the four weeks of treatment, the body weights of all the treated groups (0.01, 0.1 and 1 g/kg bw) were slightly less than the control group, although the difference was not statistically significant. Likewise, there were no statistically significant changes in ALT or AST in treated vs the control group. Histopathologically, there was very minimal and mild fatty degeneration in the portal tracts of the mid- and high-dose animals Dollah.

Studies of six weeks or longer

In a 42-day study with mice, Bensiameur-Touati administered a water extract of N. sativa seeds to female mice for six weeks to determine baseline toxicity in anticipation of a follow-up study. The N. sativa seeds were obtained from a region of southern Algeria and an aqueous extraction performed with heat and constant stirring. The extract was filtered and allowed to dry in a heated dish to a pasty consistency. The extraction yield was on average, 24%, although the extract was not otherwise characterized. For the in-life portion of the study, six- to eight-week-old female mice (average bw of 29.84 g), were randomly separated into six groups of five mice each: the control group received distilled water and the remining groups received 2, 6.4, 21, 33 or 60 g/kg bw of the extract daily, by gavage, for six weeks. At the conclusion of the study period, the animals were bled via the retroorbital sinus and sera frozen for later analysis. At termination, the animals were sacrificed with urethane and the livers and kidneys were harvested and fixed in paraformaldehyde. The sera were later tested for concentrations of urea, creatine, albumin, and hepatic function, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (AP).

Following the course of treatment, Bensiameur-Touati reported there had been six mortalities, with three in the high dose group. There were no statistically significant differences in body weights at the end of the study. There was a statistically significant increase in high dose animals compared to controls in creatinine, AST, ALT, but a decrease in alkaline phosphatase. There were no changes in urea and albumin when comparing control to treated values. The authors reported that in the liver, there were initial inflammatory changes in the 2 g/kg group, a finding which increased at the 6.4 g/kg group, along with hypervascularization and dilation of the sinusoidal capillaries. At 21 g/kg, there was total disorganization of hepatic tissue, inflammatory bodies and clear necrosis. Interestingly, at 31 g/kg, the inflammatory changes were seen to be reversing themselves and at 60 g/kg if the animal did not die on study, the liver tissue seemed to have adapted and appeared to have recovered. A similar picture of inflammation and tissue

destruction at the lower doses and recovery at the highest dose was seen in the kidney as well.

In preparation for a 56-day (8 week) dietary study, the investigators (Sultan et al., 2009), first obtained black cumin seeds from the Barani Agricultural Research Institute and indigenous to the area of the city of Chakwal, located more or less in the center of Pakistan. Fixed oil was extracted from the seeds according to an AOCS technique, using hexane as a solvent. The essential oil was obtained through steam distillation of the ground seed. Male Sprague-Dawley rats, 6 – 7 weeks old and weighing 130+10 gm were selected for study. Standard AIN-76A diet was supplied. The control group was fed diet alone, Group #2 fed a diet into which fixed oil was added at a level of 4% (approx. 2,000 mg/kg bw/day) and Group #3 fed a diet into which the essential oil was added at a level of 0.3% (approx. 150 mg/kg bw/day). The animals were allowed access to food and water ad libitum. Half the animals of each group were sacrificed at four weeks and the other half at 56 Days. At each sacrifice, blood samples were taken via cardiac puncture for hematology (erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, platelet count, erythrocytes sedimentation rate, total white blood cell and differential counts) and serum chemistry (liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) and total bilirubin, urea, total protein, albumins, globulin and A/G ratio, electrolytes and cardiac enzymes (lactate dehydrogenase), creatine kinase and its isoenzyme MB (CPK and CK-MB)) and organs harvested (heart, liver, kidneys, spleen, lungs and pancreas) which were trimmed and weighed. Although the manuscript was not clear regarding when the changes became statistically significant, from the illustrations included in the manuscript, food intake of treated animals began to lag behind controls at Week 5 and continued until the end of the study. There did not appear to be any difference in water intake between the groups. Body weights appeared to be greater in controls starting at Week 4 and steadily increased to the end of the study. There were no significant differences between treated and control animals in heart to body weight ratios. Statistical analysis of hematology and clinical chemistry values indicated no changes from control values. No histological examinations were described. The authors indicated administration of the fixed oil produced less body weight gain than controls.

In a subchronic (84-day) study, Zaoui obtained N. sativa seeds grown in northern Morocco and the seeds were authenticated by a plant taxonomist. The N. sativa seeds were powdered mechanically, and the extract was obtained by cold shocking the powered seeds in hexane. The hexane was removed under vacuum. For the in-life phase, the authors used 24 Wistar Kyoto rats weighing 200 grams each, that were divided into two groups of 12 rats each. The animals received 2 ml/kg bw distilled water or N. sativa oil, each day for 12 weeks. All animals were kept on a 12-hour dark/light schedule and fed standard lab chow ad libitum. Body weights were obtained at initiation and at 2, 4, 6, 8, 10 and 12 Weeks; samples for clinical studies were obtained at initiation, 4, 8 and 12 Weeks for hematology (erythrocytes, leukocytes, platelets, hematocrit, hemoglobin, mean globular volume; mean corpuscular hemoglobin content, mean corpuscular concentration of hemoglobin) and clinical chemistry (aspartate-aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, gamma-glutamyltransferase, triglycerides, cholesterol, high density lipoproteins, uric acid, creatinine and glucose). Blood samples for hematology and clinical chemistry were taken via the retro-orbital sinus following a 15-hour overnight fast. At sacrifice, the heart, liver, kidneys and pancreas were removed, weighed and placed into preservative. Staining techniques included H&E, collagen stain and periodic acid-Schiff stain (PAS). The results of the Zaoui studies were as follows: Body weights of treated animals were statistically significantly decreased from control values starting at Week 6 and each weighing period thereafter. There were no changes in organ weights (heart, liver, pancreas or kidneys) in treated rats at 12 Weeks when compared to controls. When compared to controls at 12 weeks, leucocyte and platelet counts were decreased as was hematocrit; whereas increases were noted in hemoglobin, mean globular volume, mean corpuscular content of hemoglobin and mean corpuscular concentration of hemoglobin. In clinical chemistries, significant decreases in treated animals were evident in alkaline phosphatase, triglycerides, cholesterol and glucose; high density lipoproteins were significantly increased. No significant histopathological changes were reported seen in any organs. The authors concluded the N. sativa fixed oil was of low toxicity.

Table16: Summary of repeated dose toxicity studies.

Substance	Source & Method of Extraction	Route, Dose & Duration	Species & Sex	Results & Comment	Reference
Aqueous suspension, not characterized	Saudi seeds were ground and added to distilled water	Gavage/ 250 & 500 mg/kg bw X 5 days	Adult albino Wistar male rats (age >24 months, weighing 250 – 300 grams)	AST, ALT, LDH and histology of liver unchanged from control.	Al-Ghamdi, 2003
Aqueous decoction, not characterized	Egyptian seeds boiled in water & filtrate administered to animals	Gavage; Group #1 -10 mL/kg X 7 days; Group #2 10 mL/kg vs controls X 14 days	Adult male albino rats	Groups #1 & #2: ↓glucose, ↑insulin, ↑ALT, ↑γGT; no change in ASP, ALP; some histopathological changes in the liver	El Daly, 1994

Aqueous decoction, not characterized	Iranian seeds, powdered seeds reflux extracted with water for 12 hours at 95°C, then extract filtered & dried; reconstituted with water	Gavage 6 grams/ kg X 14 days	Male mice	No change in BW of animals; ↓ ALP; no change in SGOT or SGPT	Vahdati-Mashhadian et al., 2005
Methanol extract, not characterized	Iranian seeds, powdered seeds reflux extracted with methanol for 12 hours at 70°C, then extract filtered & dried; reconstituted with water	Gavage 6 grams/ kg X 14 days	Male mice	No change in BW of animals or liver enzymes	Vahdati-Mashhadian et al., 2005
Chloroform extract, not characterized	Iranian seeds, powdered seeds incubated in chloroform for four days at 25°C, then extract filtered & dried; delivered in sesame oil	Gavage 6 grams/ kg X 14 days	Male mice	No change in BW of animals or ALP; ↓	Vahdati-Mashhadian et al., 2005
Aqueous decoction, not characterized	Sri Lankan seeds boiled in water for 90 minutes and filtered	Gavage 10 mL of extract X 14 days	Male Sprague-Dawley rats	↑γ-glutamyl transferase; no change in ALT, AST or ALP; no histopathological changes in liver	Tennekoon et al., 1991
Commercially obtained N. sativa oil (expressed), not characterized	Egyptian (?) seeds, expressed	Gavage; Controls, Grp II 15 mL oil/kg bw, Grp III 25 mL oil/kg bw; every other day for one month	Adult, male albino rats	Histopathological changes in kidney and liver.	Zaghlol et al., 2012
Ground whole seeds, not characterized	Indian seeds, washed, ground up and added to rat chow	Dietary 0.01, 0.1 and 1 g/kg bw X 35 days (0.1 g/kg equivalent to human exposure)	Male, Sprague-Dawley rats	High dose animals ↓creatinine; no histological changes in any groups.	Dollah et al., 2013a
Ground whole seeds, not characterized	Indian seeds, washed, ground up and added to rat chow	Dietary 0.01, 0.1 and 1 g/kg bw X 28 days (0.1 g/kg equivalent to human exposure)	Male, Sprague-Dawley rats	No changes in ALT or AST, very minimal and mild fatty degeneration in liver in mid- & high dose animals.	Dollah et al., 2013b
Aqueous extract, not characterized	Algerian seeds, seeds extracted with water with heat and constant stirring, dried to a pasty consistency and re-constituted with water.	Gavage; controls given water, test groups received 2, 6.4, 21, 33 or 60 g/kg bw daily X 6 weeks	Female, mice, 6 groups of five mice each	Six mortalities, one at 6.4 g/kg and two deaths at 21 g/kg and three deaths 60 g/kg There were no statistically significant differences in body weights at the end of the study. In high dose animals: ↑creatinine, ↑AST, ↑ALT, ↓ALP; no changes in urea or albumin. Hepatic inflammatory changes at lower doses, but seemed to resolve at the two highest doses	Bensiameur-Touati et al., 2017
Hexane extract of seeds to obtain fixed oil; essential oil obtained by steam distillation, neither were characterized	Pakistani seeds, extracted with hexane	Dietary; Gp#1 fed diet alone, Gp#2 diet + fixed oil at 4% (approx. 2,000 mg/kg), Gp#3 diet + essential oil at 0.3% (approx. 150 mg/kg bw) X 8 weeks	Male, Sprague-Dawley rats	No difference between control and test groups for: hematology (erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, platelet count, erythrocytes sedimentation rate, total white blood cell and differential counts) and serum chemistry (liver	Sultan et al., 2009

				enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) and total bilirubin, urea, total protein, albumins, globulin and A/G ratio, electrolytes and cardiac enzymes (lactate dehydrogenase), creatine kinase and its isoenzyme MB (CPK and CK-MB)), body weight, or bw to organ weight ratios. No histological examinations described.	
Fixed oil, fatty acids identified; not otherwise characterized	Morroccan seeds were ground to a powder & extracted with hexane	Oral; 2 mL/kg distilled water of N. sativa oil, daily X 12 weeks	Wistar Kyoto rats, sex not given	↓BW, no change in weight of heart, liver, pancreas or kidneys; ↓leukocytes, platelets and hematocrit; ↑hemoglobin, mean globular volume, MCHC. ↓ALP, triglycerides, cholesterol and glucose; ↑HDL. No reported histopathological changes.	Zaoui et al., 2002

Table16: Summary of repeated dose toxicity studies.

Teratogenicity/reproductive toxicity

Parhizkar et al. (2011) investigated the estrogen-like activity of Nigella sativa in ovariectomized rats using ground N. sativa in the feed and the uterotrophic assay. The investigators obtained N. sativa seeds (imported from India) and purchased at a local market in Serdang, Malaysia. The seed was identified, authenticated and voucher specimens stored. The seeds were cleaned under running tap water for 10 min, rinsed with distilled water and air dried at 40°C overnight. The seeds and pelleted chow were ground to a powder (separately) and then remixed and re-pelleted. The pellets to be made available to the animals (given the known amount the animals consume per day), the pellets will supply appropriate dose levels N. sativa. The animals were 16-week-old female albino Sprague-Dawley rats, weighing 250 – 300 grams. Forty rats were ovariectomized in order to induce menopause and were divided equally into five groups of eight animals each. Group #1 was a negative control (gavaged with 1 mL distilled water, Group #2 was a positive control (gavaged with 0.2 mg/kg bw/day with conjugated equine estrogen-CEE in distilled water), Group #3 received 300 mg/kg ground N. sativa in the diet, Group #4 received 600 mg/kg ground N. sativa in the diet and, Group #5 received 1200 mg/kg ground N. sativa in the diet. The animals were kept on study for 21 days and sacrificed under chloroform anesthesia. Body weights and uterine weights were taken and normalized to achieve relative organ weights (organ weight was divided by body weight and multiplied by 100); the uteri were preserved, sectioned and examined under H&E staining.

The authors reported the mean weight of the positive control uteri was greater than the sham treated control and although an illustration (a bar graph) visually indicated the mean weights of the N. sativa groups were greater than the negative control (but less than the positive control), the authors made no affirmative statement that the differences between the N. sativa-treated groups was greater than the negative control. Further, the illustration showed the relative uterine weights of the N. sativa-treated groups to be inversely proportional to dose; that is, the lowest N. sativa treatment exhibited the greatest mean uterine weight, and the highest dose showed the least mean uterine weight of all treated animals.

The histological examination included endometrial thickness, myometrial thickness, luminal epithelial cell height, glandular epithelial cell height, number of glands and number of blood vessels. For the histological examinations, the authors reported much the same as the uterine weights; that is, the estrogen treated animals had uniformly greater values for the aforementioned uterine morphology endpoints than the sham-treated controls and, the low to high dose N. sativa controls had values for uterine morphology that were inversely proportional to dose, such that from the highest to the lowest qualitative values were: positive control > lowest dose N. sativa (300 mg/kg bw/day) > mid-dose N. sativa (600 mg/kg bw/day) > high dose N. sativa (1200 mg/kg bw/day) > sham-treated controls (1 mL distilled water).

Genotoxicity

Nguyen obtained Nigella sativa seeds grown in three different regions in Morocco (Arfoud, Fkih ben Salah and Settat) for their series of tests. The seeds were washed, dried and ground to a fine powder, which was then homogenized and dissolved in phosphate buffered saline. Stock solutions of 180, 80 and 10 mg/ml were prepared, filtered and centrifuged to remove any insoluble debris.

The potential mutagenic and antimutagenic effects of the Nigella sativa seed extracts were determined using the Ames test with Salmonella typhimurium strain TA 98 and TA 100. To determine mutagenicity, the test solution contained 50 µL test sample and 50 µL solvent control. The positive control in the test with S9mix was 1 µg/plate 2-aminoanthracene (2AA). In the test without S9mix, the positive controls were 0.2 µg/plate 4-nitroquinoline oxide (4-NQO) for TA98, and 5 µg/plate sodium azide (SA) for TA100, respectively. None of the extracts were shown to be mutagenic, with or without S9 activation.

Nguyen followed up the Ames test with the alkaline comet assay to investigate possible genotoxicity in human C3A cells. The cells were seeded and incubated in a standard growth medium for 24 h. Then they were exposed with the test substance in concentrations of 2, 4, 9 mg/ml for the next 24 h. Ethyl methanesulfonate (EMS, 0.25 M) was used as positive control. A 50 µL of cell suspension was mixed with agarose and was loaded on the precoated slide and subjected to electrophoresis. The DNA was stained with GelRed and Vectashield, and duplicate slides analyzed automatically with a fluorescent microscope. The percentage of DNA in the comet tail was used as a measurement of DNA damage. The extract from Settat (4 mg/mL) and Arfoud (2 and 4 mg/mL) showed some DNA damaging capacity compared to control values. Co-exposure of plant extract and positive control EMS showed a statistically significant increase in DNA damage for all extracts Nguyen.

Nguyen performed a cytokinesis-block micronucleus assay according to the standard procedures, using methyl methanesulfonate (MMS) as a positive control; the cell preparation and exposures were the same as for the comet assay. After exposure and incubation, the cells were harvested and spread on slides, stained with DAPI stain (4',6-diamidino-2-phenylindole, a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA) and scored for the presence of micronuclei. To examine the cytotoxicity of a test compound, the Cytokinesis-Block Proliferation Index (CBPI) was calculated by determining the number of mononucleated, binucleated and multinucleated cells among 500 cells.

The plant extracts exhibited toxicity at 9 mg/ml, especially the sample from Settat, and to a lesser extent Arfoud. According to CPBI values toxicity at this concentration was also evident and present in the extracts from all locations. Calculated % cytostasis values were indeed all higher than 70%, lower concentrations were not toxic. The extract from Erfoud and Fkih ben Salah induced significantly higher number of micronuclei, but only at the toxic concentration of 9 mg/mL Lower concentrations showed no increased frequencies of micro nuclei, except the sample of Erfoud (2 mg/ml), although there was no dose-effect relationship, as no effect was found at the next highest dose (4 mg/mL).

In cells exposed to extract and mutagen (MMS) the extract from Erfoud significantly induced more micronuclei than MMS alone. The sample from Erfoud therefore showed co-genotoxicity except at the toxic extract concentration of 9 mg/mL. Extracts from Fkih ben Salah and Settat inversely exhibited antigenotoxicity by significantly reducing the number of scored micronuclei at the highest non-toxic concentration.

Antimutagenic effects

In the publication by Nguyen, To determine antimutagenicity, the test solution contained 50 µL test sample and 50 µL positive control. The top agar mixture was poured over the surface of a minimal agar plate and incubated for 48 h at 37°C. After incubation, the numbers of revertant colonies (mutants) in each plate were counted. A compound was considered a mutagen when the number of colonies in one or both strains was doubled compared to that found in the negative (solvent) control plates. The test extract was diluted to the final concentration of 9-4-1 mg/plate. The negative (solvent) control was PBS, as extracts were dissolved in this solvent. Antimutagenicity was expressed as the percentage of inhibition of mutagenicity induced by the positive control. The extracts did not show antimutagenic properties indeed, in some cases there was an indication of a co-mutagenic response.

A test for antimutagenic effect in the comet assay did not indicate any antimutagenic effects with any of the N. sativa extracts, instead there was a co-mutagenic response (Nguyen et al., 2019).

Franco-Ramos examined the anti-mutagenic and anticytotoxic effect of N. sativa through a micronucleus test (MNT) using the peripheral blood of six- to eight-week-old male BALB/c mice. The mice were divided into four groups with five animals per group: (1) Control (sterile water), 2 mg/kg bw X 1-day, intraperitoneal (IP); (2) N. sativa oil, 500 mg/kg bw/day X 7 days, IP; (3) Cisplatin (CP), 2 mg/kg bw X 1 day subcutaneous (SC); (4) N. sativa + CP with their respective dosage. When evaluating polychromatic erythrocytes (PCE), a biomarker of cytotoxicity, the group treated with N. sativa + CP experienced an increase in the frequency of PCE, which demonstrated the recovery of bone marrow and modulation of cell proliferation. The analysis of micronucleated polychromatic erythrocytes (MNPCE), an acute genotoxicity biomarker, showed similar frequency of MNPCE within the groups except in CP, but, in the N. sativa + CP group, the frequency of MNPCE decreased and then regulated. Finally, the frequency of micronucleated erythrocytes (MNE, Howell-Jolly bodies), a biomarker of genotoxicity, the supplementation of N. sativa oil did not induce genotoxic damage in this model. Thus, the authors concluded that N. sativa has both cytoprotective, genoprotective effects and modulates cell proliferation in BALB/c mice. Unfortunately, the N. sativa oil was not otherwise identified as the essential oil or fixed oil or an extract or pressing of the seeds, nor was the origin of the oil (i.e., the country of origin of the seeds or oil), nor any identification of the constituents (e.g., thymoquinone or fatty acids).

Cytotoxicity studies

Nguyen tested their Nigella sativa extract, in the neutral red uptake (NRU) cytotoxicity assay using human C3A cells; according to the authors, these cells are a clonal derivative of Hep G2 liver cancer cells that the authors had previously used because of their nitrogen metabolizing activity comparable to perfused rat livers and largely conserved phase I and II

biotransformation capabilities. The authors indicated the NRU test revealed that concentrations higher than 9 mg/ml reduce cell viability of human C3A cells to less than 70%. Hence, this and higher concentrations (at least for the Arfoud and Settat samples) were toxic.

Islam prepared a supply of Nigella sativa volatile oil (NSVO), by first milling the seed (of unknown provenance) to obtain the oil, followed by steam distillation and extraction of the distillate with n-hexane. Removal of the n-hexane yielded the volatile oil. The yield of NSVO, 0.4%, was similar to that reported by other authors. The NSVO was not otherwise characterized. The NSVO was serially diluted (500, 250, 125 and 62.5 µg/mL) and tested against a panel of five human stomach cancer cell lines SCL, SCL-6, SCL-37'6, NUGC-4, Kato-3 and a fibroblast line 3T6 using the MTT assay as a measure of cell viability to determine LC50 values. Vinblastine sulphate and mitomycin C (dilutions of 250, 125, 62.5 and 31.25 µg/mL) were used as positive controls. The LC50 values for the cell lines were SCL=155.02 ±10.4, SCL-6=185.77 ± 2.9, SCL 37'6=120.40 ± 20.5, NUGC-4=384.53 ± 12.1 and 3T6 fibroblast line=286.83± 45.6 µg/ml. The NSVO at 500 µg/mL showed an LC50 value of 72.73 ± 6.40 for Kato-3 cells, but the activity was not dose responsive. Vinblastine sulphate and mitomycin C showed LC50 values ranging from 48.60 ± 5.0 to 69.31 ± 7.22 µg/ml against the cell lines tested. The authors concluded that the NSVO is lethal or cytotoxic to both the cancer and fibroblast cells.

Other studies

To test the immune modulating properties of Nigella sativa volatile oil, Islam et al. (2004) prepared a supply of Nigella sativa volatile oil (NSVO), by first milling the seed (of unknown provenance) to obtain the oil, followed by steam distillation and extraction of the distillate with n-hexane. Removal of the n-hexane yielded the volatile oil. The yield of NSVO, 0.4%, was similar to that reported by other authors. The NSVO was not otherwise characterized.

Long-Evans rats were immunized to typhoid TH antigen (attenuated Salmonella typhi-H) by injection on Days 1, 21 and 28. During the period of immunization the test animals received an injection of NSVO twice a week for 30 days; the control animals (no NSVO) received soybean oil injections. At the conclusion of the immunization period, the animals were sacrificed, blood collected, and serum extracted; peripheral white cells were counted. Serum antibody titer was analyzed by agglutination tube test. The serum was diluted serially with saline (0.9%NaCl) to give 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560 dilutions. To each of the dilutions, 25 µl of TH antigen preparation was added and incubated at 50°C for 2 h, tubes were then examined to find flocculates.

NVSO significantly (P=0.002) reduced serum antibody titer to 1280 for the NSVO-treated animals as compared to the control with an antibody titer of 2560 (P=0.002) for the controls. Splenocyte and neutrophil counts in the experimental animals were found to be significantly (P<0.001) reduced compared to those in the controls, although peripheral lymphocytes and monocytes were increased significantly (P<0.001).

Human studies (Table 18)

Ligeza conducted a test of several cold-pressed oils to determine their acceptability for use in cosmetic products, in addition to an assessment of the technical qualities and aesthetic effects of the oils, the authors conducted patch tests on 23 individuals to determine possible allergenic (contact allergy) and irritating effects. The oils tested were from the following botanical sources: chokeberry seed oil (Aronia melanocarpa (Michx.) Elliott), blackcurrant (Ribes nigrum L.), elderberry (Sambucus nigra L.), raspberry (Rubus idaeus L.), apricot (Prunus armeniaca L.), tomato (Lycopersicon sp. Mill.), strawberry (Fragaria × ananassa Duchesne), broccoli seed (Brassica oleracea L. var. italica Plenck), black cumin (Nigella sativa L.), hemp (Cannabis sativa L.), safflower (Carthamus tinctorius L.), milk thistle (Silybum marianum (L.) Gaertn) and coconut (Cocos nucifera L.). The oils were not otherwise characterized. To assess for potential allergenic and irritant effects, healthy volunteers (12 females and 11 males, 18 – 60 years of age, median age 18 years) were patch tested using a small amount of oil on blotting paper which was adhered (under occlusion) to the skin of the back. Following a 48-hour interval, the blotting papers were removed, and the skin was evaluated for irritation and following an additional 48-hour interval, possible signs of an allergic reaction were evaluated. The patch test revealed only one allergic contact reaction (apricot seed oil, 4.3%), no other allergic reaction or irritation reactions were reported for the other oils (including Nigella sativa).

Soleymani conducted an efficacy test of a hydrogel containing an extract of Nigella sativa on acne vulgaris. N. sativa seeds were purchased at a local herb market in Tehran, Iran and authenticated. One kilogram of seeds was pulverized in an electric mill and extracted three times using a hydroethanol solution (80:20 v/v ethanol/water), the extract was concentrated in a rotary evaporator and condensed in a vacuum oven. The amount of active ingredient in the hydrogel was calibrated using thymoquinone and quercetin (for flavonoid content) as a standard. The hydrogel (with or without N. sativa extract) was applied to the patients faces twice daily for 60 days. Although the concentration of N. sativa extract (in thymoquinone or quercetin equivalents) was not evident, the authors did report the treatment was efficacious and that there were no adverse effects.

Latiff investigated some of the effects of twice daily administered capsules of Nigella sativa (total, 1600 mg/day) on nine urban Malaysian non-hysterectomized menopausal and perimenopausal women (45 – 65 y.o.) in a cross-over study with 12 weeks of treatment, followed by two weeks of washout and 12 weeks of placebo. The Nigella sativa was not otherwise characterized or identified. The subjects were quantitatively assessed for basal metabolic index (BMI), waist/hip ratio, fasting blood sugar, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, creatinine, total bilirubin and systolic and diastolic blood pressure. These assessments were made at baseline (prior to) and following Nigella treatment and following the 2-week washout period, baseline (prior to) and following the control (placebo) administration period. Of the clinical parameters named above BMI, total cholesterol, HDL, creatinine, total bilirubin and blood

pressure (systolic and diastolic) improved significantly from baseline (P<0.05).

Akrom and Darmawan used a standardized black cumin seed oil (BCSO) to determine the baseline effects in human subjects. The BSCO was standardized for the concentration of thymoquinone and formed into soft capsules for administration. The soft capsules each contained the test substance as follows

Table17: Content of BCSO in each 1.5 ml soft capsule.

Substance	Conc. (%)	Substance	Conc. (%)
Thymoquinone	2.72	Linolenic	0.1
Caprylic acid	0.21	Eicosanoic acid	3.15
Capric acid	0.15	Eicosenic acid	0.15
Lauric acid	0.1	Eicosadienoic acid	0.25
Myristic acid	0.18	Arachidonic acid	0.03
Palmitic acid	12.27	Eicopentanoic acid	0.03
Palmitoleic acid	0.28	Behenic acid	0.06
Heptadecanoic acid	0.1	Docoheksanoic acid	0.04
Oleic acid	0.07	Teracosanoic acid	0.02
Linoleic acid	2.85		

Inclusion criteria for study subjects were healthy men and women between 18 and 60 years, excluded were pregnant women and anyone with a history of hypersensitivity to N. sativa oil. A total of 36 subjects (10 men and 26 women), were selected and divided into three groups and given 1.5, 3 and 4.5 ml/day (Groups 1, 2 and 3, respectively) for 20 days. At a concentration of 2.72% of each capsule of 1.5 ml, the subjects received 0.0408 thymoquinone/capsule. Statistically, there was no difference in the subjects based on sex, age, body weight, BMI value, history of hypertension, education, occupation or marital status between groups. Likewise, there was no difference between groups in means of hemoglobin; erythrocyte, leukocyte and leukocyte counts, as well as differentials counts, erythrocyte sedimentation rate, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, glucose, total cholesterol, triglyceride, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, interferon gamma, T-helper cells (Th cells; CD4+), regulatory T cells (CD4+CD25+) and blood pressure. Following 20 days of administration, the subjects were analyzed for any group mean difference in the parameters cited above. The authors reported no difference between group means of any of the parameters tested. Assuming a density of 1.0 g/ml for thymoquinone, the high dose subjects received 1.2 g/day for 20 days and at a mean body weight of 61.5 kg, the NOAEL was 19.5 mg thymoquinone/kg bw/day.

Qidwai tested ground N. sativa seeds (Kalonji) in capsules (500 mg/capsule, to be taken twice daily for six weeks) to normal, healthy subjects who were checked for differences between treated and control groups for body–mass index, waist–hip ratio, blood pressure, fasting blood sugar, total cholesterol, LDL, HDL, triglycerides, serum creatinine alanine aminotransferase, caloric intake and average daily exercise (in minutes). There were 39 patients in the intervention group and 34 in control. No differences in these parameters were reported at the end of the study.

A 28-year-old man reported to a dermatology clinic with a two-day history of maculopapular eczema on the neck and spreading to the arms and back. He reported he had used a topically applied oil of black cumin (Nigella sativa). The patient had no history of allergic or atopic skin disease. Patch testing with DKG standard series and black cumin oil revealed +++ reactions to the black cumin oil. The black cumin oil was retested following dilutions of 1:100 and 1:200 and again, resulted in a response of +++. Black cumin oil diluted 1:200 was subsequently patch tested in 10 controls without any reactions.

A 31-year-old woman reported to a dermatology clinic, complaining of an eight-month history of eczema on both hands, which presented as a diffuse erythema along with vesicles, pustules and scales on both palms, spreading to the forearms. The patient complained of severe itching in the affected area. The patient had been using an ointment containing black cumin. The patient was tested with the European standard series, the ointment used by the patient and cold pressed black cumin and cumin (the latter named cumin was not further described). The patient showed ++ reactions to the ointment and black cumin only. The patient fully recovered following topical corticosteroid treatment and avoidance of the black cumin containing ointment (Zedlitz et al., 2002).

Table18: Summary of observations in healthy humans

Substance	Source & Method of Extraction	Route, Dose & Duration	Species & Sex	Results & Comment	Reference
Cold-pressed oil, only FA identified	Source of seeds not disclosed; commercially derived	Occluded patch with oil X 48 hours then patch removed and graded for irritation; after another 48 hours the areas were graded for allergic reaction	11 males & 12 females, 18 – 60 years of age	No irritation or allergy to cold-pressed N. sativa cold-pressed oil	Ligeza et al., 2016
Hydroethanol extract (80:20 v/v ethanol/ water; calibrated against thymoquinone and quercetin	Seed source was a local market in Tehran. Sees were ground and extracted three times in 80/20 ethanol/water.	Dermal; hydrogel with and without N. sativa extract applied to faces of acne patients twice daily for 60 days	36 women and 24 men (mean age 24.15 yrs)	No adverse events and mixture were deemed efficacious in suppressing some symptoms of acne vulgaris.	Soleymani et al., 2020
Whole ground seeds in capsule; not characterized	Seed source not identified	Oral; 800 mg capsules X twice a day; 12 weeks of treatment followed by two weeks of washout, then 12 weeks of placebo	Menopausal and perimenopausal women (45 – 65 yrs) cross-over study	BMI, total cholesterol, HDL, creatinine, total bilirubin and blood pressure (systolic and diastolic) improved significantly from baseline	Latiff et al., 2014
Black cumin seed oil (BCSO); standardized for thymoquinone	Seed source or BCSO source not identified	Oral; Dosed with 1.5, 3 and 4.5 mL/day X 20 days (high dose received ≈ 1.2 thymoquinone for 20 days therefore, the NOAEL was 19.5 mg thymoquinone/kg day		No difference in hematology, clinical chemistry, T-helper cells, T regulatory cells and blood pressure	Akrom and Darmawan, 2017
Ground seeds in capsules	Seed source not identified	Oral; 1000 mg/day X 6 weeks	Sex in given; 39 patients in intervention group and 34 in control group completed study	No difference between treated and control in between treated and control groups for body–mass index, waist–hip ratio, blood pressure, fasting blood sugar, total cholesterol, LDL, HDL, triglycerides, serum creatinine, alanine aminotransferase, caloric intake and average daily exercise (in minutes).	Qidwai et al., 2009
Clinical report	Seed source or oil source unknown.	Patient had skin reaction to application of oil from N. sativa	Male, 28 yo	On test, Pt responded 1:200 dilution as +++ (severe), whereas controls had no Rx at this dilution	Steinmann et al., 1997
Clinical report	Seed source or oil source unknown	Extensive eczema on hands and arms from black cumin oil	Female, 31 yo	On test, Pt responded + + to black cumin oil	Zedlitz et al., 2002

DISCUSSION

Nigella sativa L. seeds have been in use by humans for three-millennia as a food ingredient and as a medicine for a wide variety of ailments as documented in many early religious and historical texts. It is a member of the buttercup family of plants and is native to warmer areas of middle Europe (e.g., Romania, Italy), and continuing to Eurasia and the Levant; it has since been transplanted to an area stretching from Morocco to Pakistan and India to China and Malaysia. It has also been grown in areas favorable to its cultivation in Europe and North America, although India produces nearly 90% of N. sativa seed in international commerce and it is produced primarily by subsistence farmers. As a result of this long practice of cultivation, adaptation to new environments and crossbreeding, it is likely that several varieties have developed and possibly even several species from the original plants, as indicated by 90+ candidates as different species of Nigella and at least 18 recognized species.

The seeds of Nigella sativa L. have an aromatic and peppery taste and are used as a food and flavor ingredient and have been recognized as GRAS by both FDA and FEMA, although there is no publicly available record of any extracts of the seeds as having been made compliant with US regulations. FDA recognizes the seeds as GRAS for animal feed use. The Association of American Feed Control Officials notes the approval for use of N. sativa seeds by FDA and incorporates it in its Official Publication. The EFSA indicates the seeds, having been in food prior to the 1987 deadline for registration as a Novel Food, is exempt from the regulation; although the EFSA does note that extracts of Nigella sativa L. may contain alkaloids as “chemicals of concern”.

As a matter of US law, no specifications or definitions for standards of identity for N. sativa seeds have been established in

the US, nor have standards been set by the American Spice Trade Association, the European Spice Association or International Organization for Standardization (ISO), although Indian Agmark grade specifications have been published. Possible economic adulterants of N. sativa seed include seeds from other species of Nigella as well as onion seed.

As might be expected with any plant product, the constituents, including the essential and fixed oils, will vary according to variety, geography, growing conditions, the state of maturation of the seed from which the oil is extracted and even potential adulterants. For example, while thymoquinone is widely expected to be the primary bioactive ingredient, it is present in widely varying amounts (between 3 – 63.3% of total essential oil).

Several toxicity studies have been conducted with whole ground seeds and with extracts using various procedures and solvents. On the basis of the results published in the literature, the essential oil fraction has an oral LD50 > 1.0 mg/kg bw (the highest dose administered) in mice and rats; aqueous, methanol and chloroform extracts have an LD50 several grams in mice; the fixed oil portion (consisting in large part of fatty acid esters) has an LD50 equal to 28.8 ml/kg.

Longer term studies from 5 days to 12 weeks, using whole ground seeds, or various extracts produced benign results. Whole ground seeds mixed into the diet, which represents a consumption most analogous to humans, at levels of up to 1000 mg/kg bw/day to rats for up to 35 days, resulted in little change, except for a mild hepatic fatty degeneration. An aqueous suspension of ground seeds, gavaged at a level up to 500 mg/kg bw/day to rats for five days did not result in any notable changes in the liver. Aqueous decoctions (boiling) administered to rats for up to 14 days resulted in changes in glucose and liver enzymes (γ-glutamyl transferase) in some studies, but not in others; likewise, mice gavaged with aqueous decoctions, methanol or chloroform extractions did not show significant changes. Mice administered a decoction at doses as high as 21,000, 33,000 and 60,000 mg/kg bw/day of extract showed mortalities and liver enzyme changes at higher doses and, interestingly, liver inflammatory changes at lower doses, but not at the higher doses. Hexane extractions given to rats for 8 to 12 weeks in the diet, resulted in one case in no changes of a nearly a complete panel of hematological and clinical chemistry assays, but in a 12-week study, there were decreases in formed bodies in the blood, increases in others and decreases in alkaline phosphatase triglycerides, cholesterol and glucose, but an increase in high density lipoprotein.

A uterotrophic assay was conducted in Sprague-Dawley rats using ground N. sativa seeds incorporated into the diet at doses of 300, 600 and 1200 mg/kg bw/day; results were unusual, with uterine weights inversely proportional to dose. The same results of inversely proportional effect was seen in endometrial thickness, myometrial thickness, luminal epithelial cell height, glandular epithelial cell height, number of glands and number of blood vessels.

A series of mutagenicity tests were conducted with phosphate buffer-extracted ground seeds of N. sativa from different regions in Morocco. The extract was negative in the Ames test with or without S9 activation, some DNA damage was demonstrated in the Comet assay compared to controls. The cytokinesis-block micronucleus assay was positive at higher concentrations from seed of some regions, but not others. Tests to define antimutagenicity were mixed. Extracts of N. sativa seeds were reported to be cytotoxic in vitro and in vivo.

Studies conducted in healthy humans included a clinical study on the cold pressed oil and no irritation or allergic reactions were reported, although there were two clinical reports of moderate to severe exposure to skin creams containing N. sativa extracts. Other clinical studies reported an improvement in acne vulgaris following application of a gel containing an extract of N. sativa, some beneficial effects in peri-menopausal women and investigational studies with healthy adults for any physiologic changes following a regimen of ground seeds or a black cumin seed oil standardized for thymoquinone. Marginal improvement was reported in the menopausal women, but no changes for the group receiving the black cumin seed oil.

The difficulty inherent in the interpretation of these results, is that with two exceptions, none of the test materials, whole seed, ground seed or extracted seed, were the constituents ever characterized; that is, none of the investigators identify the constituents present in the test substance, despite the fact that N. sativa seeds are credited with having such profound bioactivity. In several reports, the authors attributed their findings to the presence of powerful antioxidant thymoquinone but failed to quantitate the amount of thymoquinone or the presence of any other antioxidant including, but not limited to dithymoquinone, thymol, thymohydroquinone, if in fact, the presumed or reported effect could be attributed to an anti-oxidative effect). These observations of potentially misplaced attribution are not unique to this monograph, reported that “many of the active components of [Nigella sativa] are chemically unstable and the pharmaceutical dosage forms were not standardized across the diverse clinical studies”. Further, “… the dosage forms did not conform to established good manufacturing practices (GMP) common with commercial sponsors, with respect to the assay of the active contents and to ensure that there was no significant degradation of the pharmacologically active substances during the conduct of the studies”.

Although many investigations took place prior to the warning of, investigators overlooked so many seemingly obvious factors capable of corrupting their results. That is, factors contributing to an unacceptable high level of uncertainty of the “knowns” about N. sativa seeds, include the fact that there are so many possible origins and varieties of N. sativa seeds, overlapping and confusing nomenclature, many similar seeds and opportunities for economic adulteration, variable environmental growing conditions affecting constituents, double cropping, production by marginal farmers, general lack of standards, vulnerable supply chains, and inconsistent extraction procedures – to name a few.may have said it best:

Comparison of N. sativa seeds of different origins [it was noted that] there was a significant difference in the concentration and profile of the volatile fraction in the various

samples … These observations [of quantitative differences] have considerable implications for clinical studies on the pharmacological effects of the N. sativa extracts: unless the extracts are standardised in terms of the concentration of the active principle (s), it will not be possible draw coherent conclusions from any such study.

While Margout was referring to clinical studies, the same could be said for the knowledge base of the toxicity and safety studies. If at least the major constituents of the tested substance are not identified and quantified, the study results are of little probative value [10].

CONCLUSION

Nigella sativa L. seed has a three-millennial history of use as both a food ingredient and a therapeutic agent credited with a phenomenal number of successful clinical applications. Presumptively, any botanical with such a lengthy history, widespread cultivation and growing conditions cannot always be identical in all respects; despite this logic, many investigators have taken the bioactivity, and the safety of N. sativa seeds, as an article of faith, declaring its efficacy and safe use in the absence of any quantitative data. However, in terms of food use of the whole seeds (and paraphrasing the SCOGS Expert Panel (FDA, 2018)): “There is no evidence in the available information on the seed of N. sativa that demonstrates a hazard to the public when it is used [in food] at levels that are now current and, in the manner, now practiced. It is not possible to determine, without additional data, whether a significant increase in consumption [as the whole seed] would constitute a dietary hazard.”

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