Iranian Journal of Basic Medical Sciences

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Cardio-protective and anti-cancer therapeutic potential of *Nigella sativa*

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ARTICLE INFO

Article type:
Review article

Article history:
Received: May 17, 2013
Accepted: Oct 10, 2014

Keywords:
Anti-diabetic
Cancer
Cardiovascular
Nigella sativa

ABSTRACT

Nigella sativa is the miraculous plant having a lot of nutritional and medicinal benefits, and attracts large number of nutrition and pharmacological researchers. *N. sativa* seed composition shows that it is the blessing of nature and it contains and many bioactive compounds like thymoquinone, α-hederin, alkaloids, flavonoids, antioxidants, fatty acids many other compounds that have positive effects on curing of different diseases. Several medicinal properties of *N. sativa* like its anti-cancer, anti-inflammatory, anti-diabetic, antioxidant activities and many others are well acknowledged. However, this article focuses on activity of *N. sativa* against cardiovascular diseases and cancer. For gathering required data the authors went through vast number of articles using search engines like Science direct, ELSEVIER, Pub Med, Willey on Line Library and Google scholar and the findings were classified on the basis of relevance of the topic and were reviewed in the article. *N. sativa* is rich source of different biologically active compounds and is found effective in controlling number of cardiovascular diseases and various cancers both *in vivo* and *in vitro* studies.

► *Please cite this paper as:*
Shafiq H, Ahmad A, Masud T, Kaleem M. Cardio-protective and anti-cancer therapeutic potential of miraculous herb *N. sativa*. Iran J Basic Med Sci. 2014; 17:967-979.

Introduction

Various herbal plants are being used for treatment of various ailments since ancient time. In the modern world, herbal plants are still receiving considerable attention as indicated by the annual growth of the herbal plants based industry in developed countries that is growing at rate of 7-15 % annually (1). In the developing countries, large segment of population (about 80%) uses herbal medicines for curing different basic medical problems (2). The reason behind their wide spread use is that they are considered as effective, safe, reliable, non-toxic, easily available and affordable as compared to modern allopathic medicines (3). There is a growing trend of modern research in this era to explore the therapeutic potential and medicinal uses of different plant species, *Nigella sativa* is among one of such plants. It is also regarded as miracle herb with rich religious and historical back ground (4).

N. sativa is a small shrub and annual flowering plant which belongs to the family Ranunculaceae. It bears white, yellow, pink and purplish delicate flowers containing 5 to10 petals (5). The fruit is an inflated large capsule which bears large number of black seeds when ripped, the seeds are known as black seeds or black cumin in English, Habbat el Baraka or Habbah Sawda in Arabic (6). They are commonly called as kalonji in Pakistan and India (7). *N. sativa* plant is commonly grown in Middle Eastern and Western Asian countries including Syria, Lebanon, Pakistan, India and Afghanistan (8).

N. sativa oil and seeds have been widely used to management of different diseases within centuries and regarded as important drug in traditional medical system in Asian and Middle East countries (Ayurveda, Unani, Arabic and Chinese medicines) (10, 11) and also recommended for regular use in Tibb-e-Nabwi (12).

This article is an effort to gather the published work of researchers against the cardiovascular disease (CVD) and anti-cancer activity with reference to *N. Sativa* and its active components, so to enhance our awareness about the potential health benefits of this miracle herb and to further arouse the interest of researchers to explore its health benefit and discover new novel drugs from *N. sativa* for treatment of these two notorious diseases. As these two ailments are the leading causes of deaths in low, as well as high income countries. CVDs are the chief cause of deaths worldwide (13). Low and middle income countries populations are most affected from cardiovascular diseases. It is estimated that over 80% of the CVD deaths occur in developing countries (14) and deaths due to CVD may touch a figure of

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23.3 million in next 15 years (15). Cancer is the second major cause of the deaths worldwide and account for about 8.2 million deaths in 2012 (16) that may rise up to 22 million in next 20 years (17).

Materials and Methods

The needed information was gathered using systematic literature searches on Science direct, ELSVIER, Pub Med, Willey online Library and Google scholar. Key words used for searching of data include *N. sativa*, *N. sativa* cardio-protective effect, *N. sativa* anti-diabetic activity, *N. sativa* anti-cancer activity and thymoquinone. About 400 relevant research and review articles were studied during the year 2013-2014. The literature used as reference is collected from last two decades but majority of paper used as reference are from 2000-2014.

The results of findings were interpreted and categorized based on the relevance to topic. First of all the traditional use of *N. sativa* is presented followed by the different studies that describes the effect of *N. sativa* on different health complications including cardiovascular, anti-diabetic and anti-cancer activities. Structure of different bioactive compounds is drawn and possible mechanism of action against different diseases is presented in this manuscript.

Traditional uses

N. sativa seeds and it's oil, were traditionally used from centuries to cure various ailments in different parts of world particularly Asia, Middle East and Africa (18, 19). *N. sativa* holds special importance to Muslims as Prophet Muhammad once stated that "*N. sativa* can heal every disease except death" (20). *N. sativa* seeds are commonly used as spice and food preservative and are considered to have abortifacient, anodyne, anthelmintic, appetizing, carminative, deodorant, diaphoretic, digestive, diuretic, emmenagogue, expectorant, febrifuge, galactagogue and purgative effects (1, 21). In folk medicines it is used to treat conditions and diseases like asthma, bronchitis, cough,

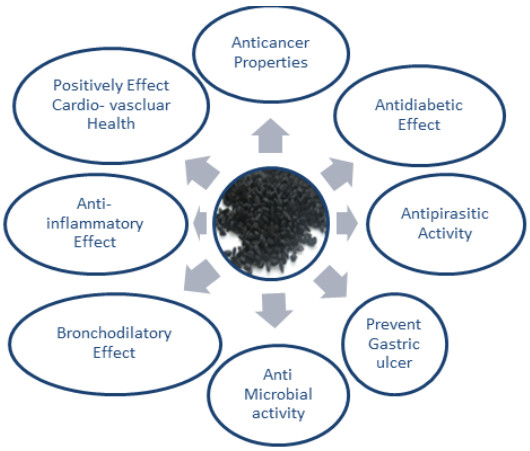

Figure 1. Health Benefits of the *Nigella sativa*

diarrhea, dysentery, dyspepsia, fever, flatulence, jaundice, paralysis, piles and other disorders related to cardio-vascular, digestive, immune, liver, respiratory and kidney systems (4, 22, 23). Its seed are rich source of many biologically active components that are known to have anti-inflammatory, anti-fungal, hypoglycemic, anti-hypertensive and anti-histaminic effects (24-27). A tincture made from *N. sativa* seed is used to cure worms and skin eruptions (4). Due to its so many beneficial health effects it is called as "Habbatul barakah" in Arabic meaning the seed of blessing (28). Some of the health benefits of *N. sativa* are depicted in Figure 1.

Chemical composition of N. sativa

N. sativa chemical composition is very diverse and consists of range of different components including carbohydrates, proteins, fats, oils, fibers, vitamins, minerals (Cu, Fe, P and Zn etc.), alkaloids, saponins and many other biologically active compounds (29). Major alkaloids identified in *N. sativa* are: pyrozol alkaloid (e.g. nigellicine and nigellidine) and isoquinoline alkaloids (e.g. nigellicimine and nigellicimine-N-oxide) as depicted in Figure 2 (30).

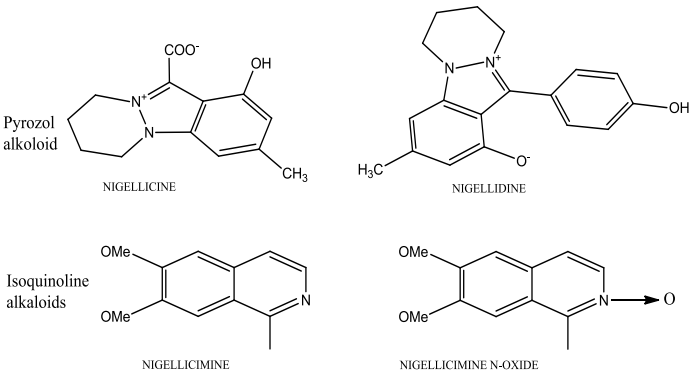

Figure 2. Alkaloids in *Nigella sativa*

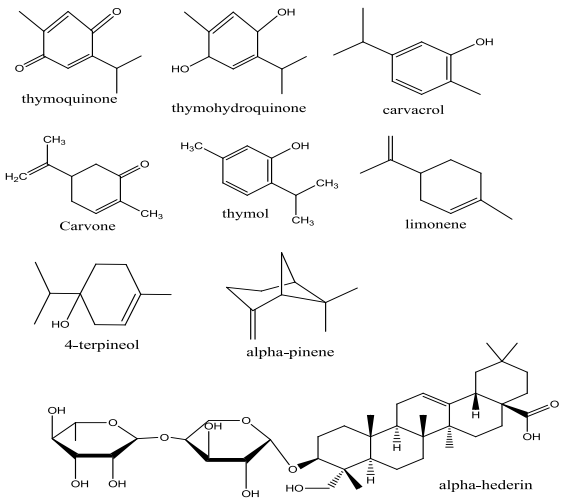

Figure 3. Biologically active compounds found in *Nigella sativa*

Range of biologically active compounds (Figure 3) found in *N. sativa* that includes: thymoquinone (TQ), thymohydroquinone, dithymoquinone (DIM), 4-terpineol, carvacrol, carvone, t-anethol, α-pinene, thymol, α-hederin, limonene and many other compounds found in trace amount (10). Most of the pharmacological properties of *N. sativa* are result of its quinine constituents (30).

The seed is also rich in fatty acids including oleic acid, linolenic acid, linoleic acid, eicodadienoic acid, arachidic acid, palmitoleic acid, palmitic acid, stearic acid and myristic acid (31, 32).

Cardiovascular Health Benefits of N. sativa

Cardiovascular diseases (CVD) refer to groups of diseases related to heart and blood vessels. The reasons of cardiovascular diseases are very diverse. Hypertensions, atherosclerosis, LDL, high cholesterol level are among the major causes of CVD (33). CVD are increasing at very rapid pace worldwide. Although older people are more vulnerable to cardiovascular diseases but these may also occur at early stages of life (34). The cardiovascular health benefits of the *N. sativ are* discussed below.

Effect of N. sativa on lipid profile

Cholesterol rich diet, oxidative stress and hypercholesterolemia can lead to development of the atherosclerosis. It is CVD in which elasticity of artery walls is reduced and they become hardened, which can lead to heart attack. Elevated serum cholesterol, LDL and triglycerides level are major cause of this ailment (35). Studies have reported that *N. sativa* has favorable effects on the lipid profile and it significantly reduces serum cholesterol LDL and triglycerides levels (7, 36-38).

In a study, to investigate the effect of the thymoquinone (TQ) consumption on the serum lipid profile of the rabbits fed upon cholesterol rich diet, results showed that TQ consumption significantly reduced total cholesterol, LDL, triglycerides and thiobarbituric acid-reactive substances concentrations, while increased HDL-cholesterol concentration (39). Similar effects were observed in other studies using *N. sativa* oil and powder (36-38, 40). *N. sativa* oil was observed to decrease serum cholesterol level by 15.5% and triglyceride level by 22 % in normal rats (41).

Promising results was also reported in human studies, it was estimated that a dose of 1 gm. *N. sativa* powder per day for a period of two months resulted in noteworthy decrease in LDL-cholesterol, triglycerides levels and increase in HDL-cholesterol level in hypercholesterolemic patients (42). Similar results were also reported in another study on the hypercholesterolemic patients in which *N. sativa* consumption was found to be associated with lowering of bad cholesterol level thus helpful in normalization of lipid profile in patients, preventing heart problems (43, 44)

Effects *N. sativa* on the lipid profile was thought to be the combined effect of various components, like TQ, sterols and flavonoids rather than any single component (25). Various mechanisms have been proposed to explain hypolipidemic effect of *N. Sativa*, including inhibition of new cholesterol synthesis or stimulation of bile acid secretion, both actions were known to reduce serum cholesterol levels (44, 45). Another reported mechanism based on antioxidant activity of *N. sativa* which prevent non-enzymatic lipid peroxidation (46).

Anti-diabetic effect of N. sativa

Diabetes mellitus (or simply diabetes) refers to group of chronic metabolic disease in which patient suffers from high blood sugar level. Untreated diabetes can lead to many serious complications particularly cardiovascular diseases and kidney failure (47). Diabetes can occur due to either pancreas were not making enough insulin (Type I diabetes mellitus) (48) or due to inability of cells to respond appropriately to insulin produced by the pancreas (Type II diabetes mellitus) (49). About 347 million people worldwide are suffering from diabetes; and the numbers are continuously increasing (14). Around 80% of the diabetic deaths occur in developing and low income countries (15). Many researchers have reported that *N. sativa* has anti-diabetic and hypoglycemic activity.

Oxidative stress is thought to play an important part in the pathogenesis of the diabetes mellitus, as oxidative stress can decreases the efficiency of pancreatic β cell and in turn effects the insulin production (50). *N. sativa* and its components are effective against diabetes as they decrease oxidative stress and thus preserve the pancreatic beta cell integrity (51). The protective effect of *N. sativa* seeds/oil on the pancreatic β cell was demonstrated in number of studies, in which it was reported that no ultra-structural changes in pancreatic cells occur

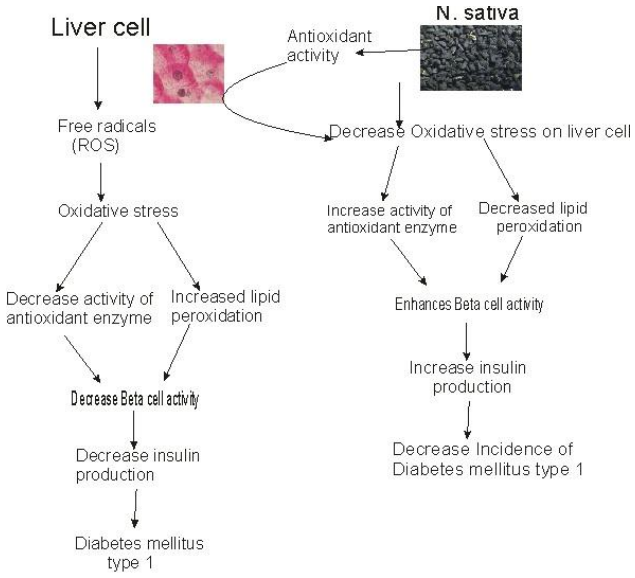

Figure 4. Mechanism of anti-diabetic effect of *N. sativa*

in STZ induced diabetic mice's which received *N. sativa* seed /oil treatment while Islet cell degeneration occur in mice's which do not received *N. sativa* seed/oil treatment (52-55). Antidiabetic mechanism of N sativa is elucidated in Figure 4.

N. sativa oil and aqueous extract are equally good in controlling serum glucose and insulin response. Positive effect of the *N. sativa* extract/oil intake on the parameters like serum insulin , super dismutase (SOD), serum glucose and malondialdehyde (MDA) levels were demonstrated in number of studies (53, 56-59). TQ content of *N. sativa* were considered as the major constituent responsible antidiabetic activity of the herb (60-62). It was found that the effectiveness of *N. sativa* in diabetic patients is further increased if it is used along with α- lipoic acid and L-carnitine. The combination of all three component was shown to have a great impact on carbohydrate metabolism in STZ induced diabetic rats and their consumption was found to enhance insulin production and C-peptide level (63).

Effect of N. sativa on diabetes type II

Diabetes type II accounts for more than 90% of all the diabetes cases (49). A study planned to evaluate the potential effects of the TQ on the activities of the two main enzymes (i.e. hexokinase and glucose 6-phosphate dehydrogenase) involved in carbohydrates metabolism in rats in which diabetes was induced by injecting STZ-nicotinamid. The results showed that TQ consumption (at the rate of 80 mg/ kg body weight) is effective against diabetic conditions and this amount also restore the activities of enzymes near to normal thus enhancing glucose consumption by the tissues and reducing the risk of type-II diabetes (64). To show the effect of *N. sativa* on the insulin resistance syndrome a study was planned with sixty patients. The patients were divided into two groups of thirty patients each. Patients of the both groups were advised to take a fixed dose of statin and metformin tablets for six weeks period. The patients of second group were additionally given 2.5 ml of *N. sativa* oil two times a day during the therapy. The results showed more improvement in serum cholesterol and fasting blood glucose of the patients of group 2 as compared to group 1 thus indicating therapeutic potential of *N. sativa* against insulin resistance syndrome (62).

In a similar study *N. sativa* seeds were used as adjuvant therapy in patients suffering from type II diabetes mellitus along with their basic medication. The subjects were divided into 3 different dose groups and were given 1, 2 and 3 gm. *N. sativa* seeds / day for period of 3 months. Results showed that dose of 2 gm. per day resulted in noteworthy reduction in fasting blood glucose level, glycosylated hemoglobin (HbA1c) and cause increase in β-cells functionality. 1 gm. per day dose resulted in improvement in all parameters but that was not statistically significant while dose of 3 gm. per day doesn't cause any additional benefit as compared to 2 gm. per day dose. So the dose of 2 gm. *N. sativa* seed / day can be beneficial adjuvant therapy in type 2 diabetes mellitus (65).

Endothelial dysfunction

It is a pathological disorder of the endothelium that play role in the pathogenesis of numerous cardiovascular disorders and can result from hypercholesterolaemia, hypertension, obesity, septic shock, diabetes and smoking as well (78, 79). In a recent study, to determine the result of TQ on the age related endothelial dysfunction, it was found that TQ recovers endothelial function at least in part, through inhibition of oxidative stress and regulation of the angiotensin system (79).

Effect of N. sativa consumption on the blood pressure and heart rate

Different studies showed that *N. sativa* positively effects the elevated heart rate and blood pressure and it consumption is associated with lowering of elevated heart rate and blood pressure (80-84). Consumption of *N. sativa* was found effective in normalizing the elevated heart rate of the cadmium treated rats (81). While in another study *N. sativa* was found effective in reducing the diabetes-induced disturbances in heart rate of the rabbits in which diabetes was induced by injecting alloxan (84).

The human study designed to access the effect of *N. sativa* on the blood pressure of the patients suffering from mild hypertension, shows that *N. sativa* extract consumption favorably effects the elevated blood pressure (85). Similar results were found in another human study in which Qidwai, *et al* (86) reported that *N. sativa* seeds have favorable effect on the high blood pressure.

Table 1. Effect of *Nigella sativa* on lipid profile and its ant diabetic effects

Study objectives	Results	References
To evaluate the hypolipidemic effects of NS in menopausal women	Significant improvement in the lipid profile was observed in menopausal women	Ibrahim, *et al* (67)
To investigate the effect of the poly herbal formulation (NS sugar powder) on diabetes and serum lipid profile	Significant reduction in fasting blood glucose and increase in HDL and decrease in TG was observed in Wistar rats	Alam, *et al* (69)
To study the effect of NS on lipid profile of the hypercholesterolemic rabbits	NS positively effects all the lipid variables in rabbits	Asgary, *et al* (70)
To explore the effect of NS on hypolipidemic and hypochlesterolemic condition	Significant reduction in the TG, TC and LDL-c was observed in rats	Ali, *et al* (71)
To study the effect of NS on hypertriglyceridemia	Significant reduction in serum TG level was observed in human	Nasir, *et al* (72)
To study NSO treatment effect upon the regeneration of pancreatic islets of langerhans in diabetic rats	Positive effect on the regeneration of the islets of langerhans was observed in rats	Sobhi, *et al* (76)
To evaluate the repairing ability of the NSO on the damaged pancreatic tissue in diabetic rats	Positive effect was observed on the morphology of the Langerhans islets in diabetic rats	Hmza, *et al* (77)
To examine the anti-diabetic potential of the NS fixed oil and NS essential oil	Reduction in MDA level and antioxidants damage and modulation of lipid profile was observed in rat	Sultan, *et al* (66)
To evaluate the anti-mutagenic effect of NS on the experimentally induced diabetes type – II	NS decreased the frequency of micronuclei in the erythrocytes of bone marrow and enhanced the antioxidant status in the treated diabetic Wistar rats	Sheikh, *et al* 68)
To evaluate the effect diet fortified with NS and Fenugreek on diabetic rats	Positive effects on the serum Glucose and lipid profile, thyroid hormones, kidney and liver functions was observed in rats	Mahmoud (73)
To study the effects of NSO and TQ on cardiovascular risk parameters in experimental hyperlipidemia	High antioxidant activity of NS and protective effect of NS was observed in rats	Shafeeque (74)
To Study of the effects of NS hydroalcholic extract on glucose concentrations in diabetic rats	NS extract cause significant reduction in FBG level and protect the great deal of the pancreatic islet cells of rats	Alimohammadi, *et al* (75)

MDA malondialdehyde, NS *N. sativa*, NSO *N. sativa* oil, HDL high density lipoprotein, TC total cholesterol, TG Triglyceride, LDL-c low density lipoprotein, FBG fasting blood glucose

Anti-cancer activity of the N. sativa

Cancer is a rapidly growing health problem and posing a serious challenge to health professionals and researchers. American cancer society has reported that more than 0.5 million deaths occur due to cancer during 2013 in USA alone and the numbers are really threatening in under-developed world (87). With the wide spread and devastating effect of cancer and its high economic load, there is need for identifying natural and cheap products with anti-carcinogenic activity. Many studies depicts that risk of cancer occurrence can be reduced by the consumption of many vegetables and fruits (88-90). *N. sativa* is one of them who showed promising anti-cancer activity in number of studies (10, 62, 91-94).

Ibn-Sina (428 Hijri) was aware of the antitumor effect of the *N. sativa* and was probably the first known physician who used *N. sativa* in the tumors treatment (10). The anti-cancer effect of *N. Sativa,* with regard to modern time was perhaps first revealed when improvement in activity of natural killer cells was detected in cancer patients who were receiving multimodality immunotherapy program in which *N. sativa* was one of the element (95). Later on, number of studies was done by many researchers to study the anti-cancer effect of *N. Sativa* seed and its extracts using both *in vivo* and *in vitro* models. Anti-cancer activity of *N. sativa* against different types of cancers is discussed below.

Lung cancer

Lung cancer is the leading cause of cancer deaths worldwide. It caused 1.59 million deaths in 2012 i.e. about 20% of total cancer deaths (16), while American cancer society estimated that of all the cancer deaths in 2014 about 27% will occur due to lung cancer (87).

The diet supplemented with *N. sativa* and honey was shown to have protective effect against lung, colon and skin cancers (96). *N. sativa* seed extract showed cytotoxicity against number of cancer cell line including Lewis lung sarcoma (LL/2) (97). Kumara and Huat (98) demonstrated *N. sativa* extract exhibit anti-cancer activity against the LL/2 tumor cell line that are subcutaneously implanted in BDF1 mice. TQ,

extracted from the *N. sativa* seed, at concentration of 100 µM showed significant anti-cancer activity against the lung cancer cell line and demonstrated to inhibit cancer cell proliferation by about 90% (99).

N. sativa oil showed significant inhibitory effect against the human lung cancer cell line A-549 with IC_{50} value of 43 µg/ml (100). Later on in a recent study, Al-Sheddi, *et al* (101) also reported that *N. Sativa* oil and seed extract significantly reduces human lung cancer cells viability and alter the cellular morphology of A-549 cells in a concentration dependent manner.

Breast cancer

Breast cancer is the most common cancer in the woman's and caused more than 0.5 million deaths in 2012 (16). It is the number one cause of the cancer deaths in women's of less developed countries while second major cause of the cancer deaths in woman's of developed countries (102).

In a study, the effect of the aqueous and alcoholic extracts of *N. Sativa* on MCF-7 (breast cancer cell line) was accessed and the results showed the *N. Sativa* extracts are effective in inactivating MCF-7 cells (103). In another study MCF-7 (breast cancer cell line) was exposed to the aqueous and alcoholic extracts of *N. Sativa* in combination with the H_2O_2 (oxidative stressor). Survivability of the MCF-7 cells was measured under different concentrations and combinations using standard cell culture technique and results showed that *N. sativa* extracts alone or in were combination with H_2O_2 are effective against MCF-7 cells and affect their survivability and can provide a promising treatment for breast cancer therapy (104).

To evaluate the protective potential of the melatonin, retinoic acid and *N. sativa* seeds against breast cancer 7,12-di-methylbenzene (α) anthracene (a well-known carcinogenic substance that induce mammary carcinoma in rats) was injected in rats. At the end of study period, serum and tissues of animals were evaluated for the factors like markers of tumorigenicity, endocrine derangement, apoptotic changes, and markers of oxidative stress. Then results showed that frequency of mammary carcinoma was quite low in rats (33%) which start receiving melatonin, retinoic acid and *N. sativa* treatment 14 days before the intake of DMBA as compared to rats which received treatment after the intake of DMBA (56%) while rats which do not received any treatment showed highest frequency (60%). The results showed that melatonin, retinoic acid and *N. sativa* are effective in reducing the carcinogenic effects of DMBA (105). Similar anti cancerous results were reported for TQ derivative against MCF-7 breast cancer cell line (106).

In a more recent study, the anti-cancer activity of the supercritical-CO_2 extract of *N. sativa* against MCF-7 breast cancer cell line was investigated and results showed that supercritical-CO_2 extract of *N. sativa* have significant anti-proliferative activity against MCF-7 cells and can be used to treat breast cancer (107).

Colon cancer

Colorectal cancer caused more than 0.69 million deaths in 2012 (16) and it is second major cause of cancer related deaths in US (87). To evaluate the preventive effect of *N. Sativa* oil on colon cancer aberrant crypt foci were induced in Fischer rats using 1,2-dimethylhydrazine. The rats were divided into four groups, first group served as a control group, second group received oil at post-initiation stage, third group received oil at the initiation stage while fourth group received 0.9% saline and oil from beginning till end of study. At the end of study i.e. 16 weeks, rats were sacrificed and results showed that *N. sativa* oil treatment in the post-initiation stage (group two) significantly reduced the total number of aberrant crypt foci however, treatment at initiation stage (group three) did not produce significant inhibitory effect. So it was concluded from the study that *N. sativa* oil has the potential to prevent colon carcinogenesis in the post-initiation stage (108). Gali-Muhtasib, *et al* (109) studied the effect of TQ on the HCT-116 (colon cancer cells line) and found that TQ is effective against colon cancer cells and trigger apoptosis in colon cancer cells in time and dose dependent manner thus inhibits the growth of cancer cells. However, TQ was not found effective against the human colon carcinoma cell line HT-29 in a study by Rooney and Ryan (110).

Renal cancer

A study was designed to evaluate the therapeutic effect of the *N. sativa* against ferric nitrilotriacetate (Fe-NTA) induced renal carcinogenesis in Wistar rats. Fe-NTA induction caused number of changes in the normal metabolic processes of the kidney. A dose of 50 and 100 mg *N. sativa* crushed seeds / kg body weight showed significant restoration of normal metabolic processes thus preventing the cancerous effect of Fe-NTA (111) In a recent study the anti-cancer activity of the *N. sativa* seed hydro-alcoholic extract was evaluated against the human renal carcinoma cells and results shows that *N. sativa* seed extract significantly inhibit the growth of human renal carcinoma cells (112).

Anti-cancer activity of the N. sativa decoction

To evaluate the anti-carcinogenic potential of the decoction, composed of *N. sativa* seed, *S. glabra* rhizome and *H. indicus* root, hepato-carcinogensis was induced in male Wistar rats using dimethyl nitrosamine. The rats were given decoction after the initiation of carcinogenesis for 10 weeks, results showed significant reduction in the number and area of dimethyl nitrosamine mediated glutathione S-transferase placental form positive foci, number of

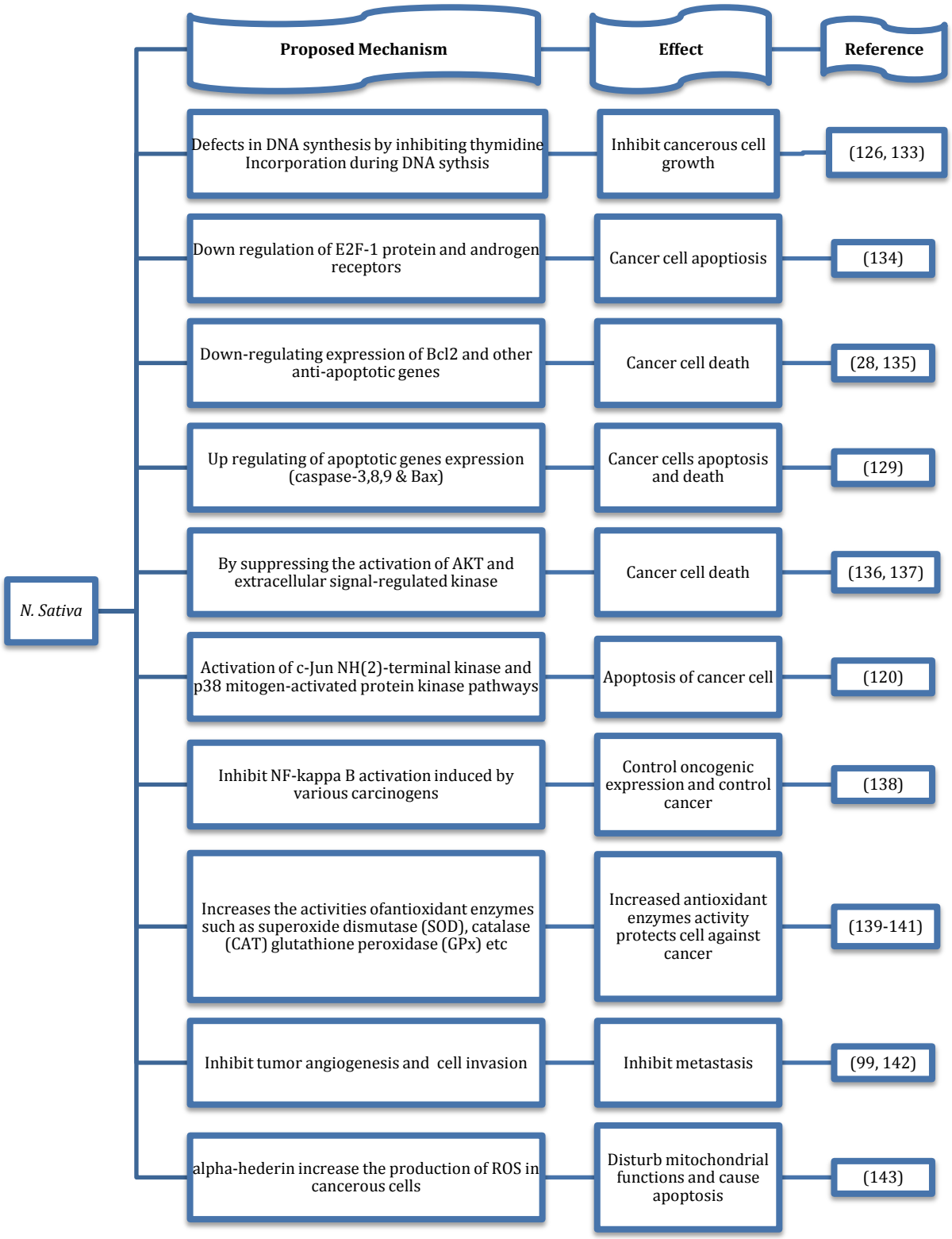

Figure 5. Different proposed mechanisms for anti-cancer activity of *N. sativa*

cells/cm^2 of foci and the staining intensity of foci in the liver as compared to controls (113). Later on, same decoction was shown to have the protective effect against dimethyl nitrosamine mediated carcinogenic changes in a study of 16 months (114). The anti-cancer effect of the same decoction was evaluated against the human hepatoma (HepG2) cell line and it was found that decoction has strong dose dependent anti-cancer activity against HepG2 cancer cell line (115). Anti-carcinogenic effect of the same decoction was further evaluated by the Samarakoon, *et al* (116) who studied the anti-cancer effect of the

decoctions made by aqueous and ethanol extracts of the *N. sativa*, *S. glabra* and *H. indicus* against human hepatoma (HepG2) cell lines using MTT and SRB assays. Results depicted that both the extracts showed strong cytotoxicity to HepG2 cells. However, aqueous extract showed quite higher cytotoxic potential as compared to the ethanol extract.

Miscellaneous studies

Delay in the onset of papilloma and reduction in their number per mouse was observed, in mice in which skin cancer was induced by application of croton oil, by the topical application of *N. sativa* extract (117). Proliferation and apoptosis in pancreatic ductal adenocarcinoma (PDA) cells was observed by the administration of TQ (118) Anti-cancer effect of TQ against the pancreatic cancer was also demonstrated by other researchers (119, 120). Shafi, *et al* (121) studied the effect of *N. sativa* organic (Hexane, methanol and chloroform) extracts on the human epithelial cervical cancer (HeLa) cell line and found that all the three extracts induced apoptosis in HeLa cells and effectively killed them. Hasan, *et al* (122) found that *N. sativa* extract caused 88.3% inhibition in proliferation of the SiHa (human cervical cancer cells line) at a concentration of 125 µl/ml.

Thymoquinone (TQ) anti-cancer activity

Probably cytotoxic activity of the TQ was first reported against Ehrlich ascites carcinoma, Dalton's lymphoma ascites and Sarcoma-180 by Salomi, *et al* (123). Later on TQ and DIM, both extracted from *N. Sativa*, were reported to have cytotoxic activity against several human cancer cell lines, that were resistant to etoposide and doxorubicin anti-cancer drugs (124). Soon after that, TQ was found effective against benzo-(α)-pyrene induced forestomach carcinogenesis in rats whose consumption (0.01% in drinking water) had shown to reduce the onset and multiplication of the benzo-(α)-pyrene induced forestomach cancer (125). Likewise, antitumor potential of the TQ was investigated against the 20-methylcholanthrene induced fibrosarcoma in mice and results showed that consumption of TQ, a week in advance and after 20-methylcholanthrene treatment, can considerably delay the onset of fibrosarcoma tumor (126).

It was reported that TQ showed antitumor activity against the SW-626 colon cancer cell line which was equivalent to 5-fluorouracil a well-known drug used in treatment of colon cancer (127, 128). Anti-cancer activity of TQ was also observed against the human leukemia (HL-60) cells (129). Furthermore, TQ also showed significant antitumor activity against hepatocellular carcinoma (HepG2) cell line in a dose-dependent manner (130, 131). In another study, TQ is showed to inhibit growth of the panel of human colon cancer cells (Caco-2, DLD-1 HCT-116, HT-29 and LoVo), without any cytotoxic effect on the normal human intestinal FHs74Int cells (132).

Possible mechanisms of N. sativa anti-cancer activity

Anti-cancer activity of any compound is due to two main reasons i.e. it either kills cancer cells or hinder any alteration in the genetic material of the normal cells. Several mechanisms have been proposed for the anti-cancer activity of the *N. sativa* that are summarized in Figure 5.

Conclusion

N. sativa is really a seed of blessing and proved to provide protection against the two most notorious ailments i.e. cancer and cardiovascular health problems. It is rich in different phytochemicals and nutritionally essential components. Health benefits of the *N. sativa* seed, oil and extracts have been shown in both *in vivo* and *in vitro* types of studies. In detail research of this herb and chemical modifications in the molecular structure of *N. sativa* active components can lead to the discovery of many novel medicines. *N. sativa* can also be used in combination with already recognized drugs. We hope that this article will increase the awareness and the interest of researchers to investigate the potential health benefits of *N. sativa*.

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