



Black Seed (*Nigella sativa*) Possess Bioactive Compounds Act as Anti-*Helicobacter pylori* Agent

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ABSTRACT

Gastrointestinal associated problems are physiological processes effects almost every individual at some stage of their life. Among the various plants studied previously *Nigella sativa* possess numerous therapeutic properties including its anti-ulcer potential. This seed carries significant anti-ulcer properties arbitrated by antimicrobial activities specifically against gastric damage induced by *Helicobacter pylori*. Evidence is available supporting the utilization of *NS* and its bioactive components in a daily diet for health improvement. This review is envisioned to emphasis on the curative role of *NS* and to provide an evidence for being a functional food to protect from a range of malaises. An attempt is also made to emphasize aspects that need further investigations for it to be use in clinics in future.

Key words: *Nigella sativa*, Black seed, Gastric ulcer, *Helicobacter pylori*

INTRODUCTION

Black cumin (*N. sativa* L.) belongs to the family Ranunculaceae. It is an annual flowering plant, native to Southwest Asia. It grows 20-30 cm tall, with finely divided, linear leaves. The flowers are usually pale blue and white, with 5-10 petals. The fruit is a large inflated capsule composed of 3-7 united follicles, each containing numerous seeds [1, 2]. The seeds of *N. sativa* commonly known as black seed, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The seeds of this plant are the most extensively studied, both phytochemically and pharmacologically. The aqueous and oil extracts of the seeds have been shown to possess antioxidant, anti-inflammatory, anticancer, analgesic and antimicrobial activities. Thymoquinone (TQ), the

most abundant constituent of black seed essential oil, has been shown to be the active principle responsible for many of the seed's beneficial effects. The medicinal potential and therapeutic values of some of the individual components are present in the extracts of the seeds [3].

N. sativa L. is said to be the universal remedy. It has an amazing healing power and its greatness has been recorded in the Hadith stating that "it could cure any diseases except death". Most of the earlier studies on *N. sativa* involved the use of either the seeds or the oil extracted from it. Such as, the ethanol extract of *N. sativa* seeds has been shown to possess antitumor activity as well as lifespan expanding activity in mice bearing Ehrlich as cites tumor cells by Musa and co-workers [4]. The best extracting solvent was determined to be used for black seed oil, in terms of time and most importantly its yield. Rotary Evaporator employed

with Ultra Sonic Bath is used in extraction process. Time and heat is chosen as the parameter to manipulate and several trials is carried out [5].

According to the common practices of 'evidence-based herbal medicine' the bioactive constituents of the volatile oil of black seed (54 per cent) were identified by El-Dakhkhany 1963, showing that TQ(I) or, in short, TQ was the main active constituent of volatile oil of the black seed although it is accompanied by other analogous compounds such as Thymol (II) and TQ dimer named as Dithymoquinone (TQ₂, III) Furthermore, traces of alkaloids were also found belonging to two different types: isochinoline is represented by nigellimine and nigellimin-Noxide and pyrazol includes nigellidine and nigellicine [7]. Subsequently Morikawa et al. 2004 isolated new dolabellane-type diterpene alkaloids, Nigellamines A(3), A(4), A(5), and C, from the methanolic extract of black cumin. Afterwards, two new aliphatic compounds were separated from hexane extract of *N. sativa* [9]. The compounds were characterized as 16-tricosen-7-ol-1 and 6-nonadecanone-2. More recently, Mehta et al. (2009) identified new saponin from its ethanolic extract. Black cumin seed contains fixed and essential oil; health claims are often attributed to functional ingredients present in them [11].

Bioactive components of *Nigella sativa* L.: Black seed extracts have been literally well characterized with organic compounds including steroids, essential and non-essential fatty acids, flavonoids, carbohydrates, alkaloids etc. Some new extraction techniques can be summarized, including supercritical extraction techniques (SFE) and microwave assisted extraction (MAE). Beside the classical method of hydrodistillation (HD), usually employed for the isolation and screening of unidentified new compounds [12]. Different oil extraction methods from *N. sativa* has been in practice while microwave extraction method proved to be useful in producing higher quantities of oil and gave best results regarding consumption of less time for extraction process. One important aspect of this technique rather imparting green effect on environment thus useful in reducing pollution [13]. According to a scientific analysis the compositional properties of Black seed have been illustrated i.e. ash, moisture, oil, proteins, and total carbohydrates contents in the range of 24.9-40.0%, 3.7-8.0%, 22.2-39.35%, 20.75-32.1% and 3.74.7%, respectively [14,15]. Its medicinal potential has been ascribed to the active constituents that are generally concentrated in essential or fixed oil. The essential oil of Black Seed contains a lipid proportion containing fat-soluble vitamins, essential fatty acids and special

amounts of volatile components, while its essential oils are composed of only volatiles [16]. The bioactive components of the volatile oil of *N. sativa* (54%) were discovered in relation to the conventional techniques of 'evidence-based herbal medicine, presenting that Thymoquinone (TQ) was the core bioactive component of *N. sativa* volatile oil even though it is complemented by additional corresponding components such as Thymol (II) and Thymoquinone dimer termed as Dithymoquinone (TQ₂, III). High Performance Liquid Chromatography (HPLC) separation of the *Nigella sativa* oil has been practiced using mobile phase of water-methanol-2-propanol (50:45:5 %v/v) isocratically. While nigellone is a carbonyl polymer of TQ isolated from Black seed. The polymer is the bioactive source of the plant usually preserves much of the pharmacologic characteristics of TQ and found to be less toxic [17]. TQ has the mechanism of action of pro-apoptotic on proteins of prostate cancer cells and on cell regulatory cycles [18] and chemically belongs to the class of compound such as 2, 5-di-substituted benzoquinone, contains isopropyl groups and methyl at C-5 and C-2 positions, respectively [19]. A number of biochemical properties of TQ generally derived from its antioxidant attributes. *Nigella sativa* contains essential amino acids among them 15 are abundantly present. Which are required numerous bodily functions in order to sustain health life. The key minerals components of black seed are potassium, iron, calcium and sodium. Black seed is high in essential fatty acids particularly Linoleic acid containing omega 6 fatty acid exhibit anti-cancerous characteristics. According to similar studies it has been proved to be useful in treatment of disorders like skin cancers and cystic fibrosis, likewise two different types of alkaloids such as pyrazol comprises nigellidine and nigellicine and isochinoline is characterized by nigellimine and nigellimin-N oxide [20].

Latest research informed that thymoquinone produces detrimental effects by overwhelming androgen receptor and E2 F-on cell proliferation of several hormone-refractory prostate cancer and linings of cancerous cell [21]. The black seed is composed of fixed oil carries functional importance of antioxidant being rich in volatiles (0.40-1.50%); contain 46% monoterpenes and 18.4-24% thymoquinone [22]. Two new aliphatic compounds 6-nonadecanone-2 and 16-tricosen-7-ol-1 were extracted from black seeds using hexane as an extraction solvent, [23]. Black seed have been explored as a good source of selenium and polyphenols. The ethanolic extracts of *Nigella sativa* produce saponin contents, [24]. Correspondingly, the concentrations of selenium, DL- γ -tocopherol and vitamin A (all-trans-retinol)

in Black seed are found as 9.027, 0.177, 5.427, 0.277mg/kg seed, respectively [25].

The *Nigella sativa* comprised of Fat-soluble vitamins, which are estimated as more than 0.2% of total black seed oil components. "The black seed exhibits anticancer activity due to containing abundant sterols in it, especially beta-sitosterol. The amount of β -carotene is about 593 μ g/g of oil. One gram of black seed fixed oil comprises of 40, 284, 48, 225 μ g of β -Tocopherol, α -Tocopherol, d-Tocopherol and γ -Tocopherol. The essential component like vitamin E, vitamin A and selenium, are essential nutritional constituents necessary for conducting a healthy pattern of life [26, 27].

Nigella sativa seeds restricts DPPH radical development and mean values for IC₅₀ (μ M) was found to be 515 \pm 20.1mg/mL [28]. Free radical killing activity of DPPH stayed in the range of 60-80% and 70-90% and for 5mg/mL of Black seed oil using water and methanolic extracts, respectively [29]. The methanolic (80%) and aqueous extract of Black seed resulted in marked inhibition of DPPH radicals with IC₅₀ (mg dry wt) of 1.25 \pm 0.2 and 2.79 \pm 0.20, respectively [30]. Rich phytochemistry of black seed has the capacity to inhibit free radicals destruction activity by increasing the oxidative stress on the cells.

Antimicrobial Properties of *Nigella sativa*: A shocking increase in bacterial resistance to prevailing agent's demands that improved efforts should be made to pursue antibacterial agents operational against pathogenic bacteria resistant to existing antimicrobials. Crude extracts and essential oil of these seeds possess antibacterial activities against several bacteria [31, 32, 33]. *Nigella sativa* L. (black seed) is an herbaceous plant, used for many times ago for the treatment of various ailments, including infectious diseases. However, the *N. sativa* extracts are effective against medically important actinomycete bacteria had not been studied. Aerobic actinomycetes notably *Nocardia*, *Gordonia*, *Mycobacterium*, *Nocardia* and *Rhodococcus* are known to have tough cell wall due to the mycolic acids contents and consequently they are resistant to many antimicrobial agents [34]. Recently an increase in the incidence of human diseases caused by aerobic actinomycetes can be attributed to the better clinical microbiological awareness, improved selective isolation and identification procedures and the extensive use of immune suppressive agents [35].

The *In-vitro* effect of aqueous extract of *N. sativa* seeds on nitric oxide production by murine macrophages was studied by pre-incubating them with the extract followed by activation with *E.*

coli lipopolysaccharide. Nitric oxide production was measured after 24 hours spectrophotometrically. The plant extract caused a dose-dependent decrease in nitric oxide production. Moreover the boiled fraction of the extract resulted in a dose-dependent inhibition of nitric oxide apparently comparable to that of the whole extract. The study revealed that the aqueous extract of *N. sativa* seeds exhibits an inhibitory effect on nitric oxide production by murine macrophages and the active component(s) is/are non-protein in nature. In view of the fact that nitric oxide is a pro-inflammatory mediator, this study validates the traditional use of the *N. sativa* seeds for the treatment of rheumatism [36].

Methicillin Resistant *S. aureus* (MRSA) is one of the commonest pathogens encountered in clinical as well as laboratory practice. It has become a major health problem worldwide. Alternate new antimicrobials/agents are urgently needed to overcome this problem. MRSA resistance to various anti-staphylococcal agents. *N. sativa* commonly known as black seed (ethanolic extract) contains the anti-staphylococcal activity. A disc diffusion method was used to assess the antibacterial activity. *S. aureus* was used as the standard reference strain. All tested strains of MRSA were sensitive to *N. sativa* extract at a concentration of 4 mg/disc while the extract had an MIC range of 0.2–0.5 mg/ml. *N. sativa* has inhibitory effect on MRSA evaluated by [37].

Treatment of *H. pylori* infection with antimicrobial agents can lead to the regression of *H. pylori*-associated disorders. Antibiotic resistance against *H. pylori* is increasing, and it is necessary to find new effective agents. *N. sativa* seed, a commonly used herb, possesses *In vitro* anti- *H. pylori* activity. The efficacy studies of *N. sativa* were conducted in eradication of *H. pylori* infection in non-ulcer dyspeptic patients [38]. Most of the bacterial pathogens are resistant to existing synthetic antibacterial agents demanding an increasing effort to seek effective phytochemicals as antibacterial agents against toxic pathogens. The antibacterial activities of the extracts were investigated by the agar dilution method against Gram-positive bacteria (*B. cereus* F 4810 and *S. aureus* FRI 7 22) and Gram-negative bacteria (*E. coli* MTCC 108 and *Y. enterocolitica* MTCC 859). SCCO₂-1 (120 bar/40°C) extract showed effective growth inhibition than conventional solvent extracts against all the tested bacteria [39].

Antiulcer Properties of *Nigella sativa*: An elevated incidence of *H. pylori* infection found in Pakistan mainly for dyspeptic patients. Therefore a high prevalence of *H. pylori* infection has been noticed providing a warning sign of its environmental risk related factors. The prevalence

of *H. pylori* in children population was assessed. Only a few of children are infected during childhood, but the prevalence of infection rises in proportion to age during adult life. The target population was from various schools of Islamabad, apparently not showing any gastritis symptoms. The presence of *H. pylori* confirmed by a non-invasive technique of ^{13}C urea breath test (UBT) was used. A non significant difference has been observed on an overall gender basis e.g, 72.3 per cent of apparently healthy children were carrying the *H. pylori* bacterium and the prevalence was 70.3 per cent and 74.0 per cent in girls and boys respectively [40]. The non-invasive ^{13}C urea breath test (UBT) was used to investigate the frequency of *H. pylori* infection in asymptomatic individuals and its association with gender and age was studied. An increase with age in both genders was found significantly higher prevalence in adolescents and adults. Moreover, a non-significant difference was observed between the prevalence of *H. pylori* infection in males and females of all age categories [41]. The prevalence of *H. pylori* infection in a community is related to the factors like acquisition of infection with *H. pylori*, loss of infection and the prolonged survival of the bacterium in the stomach [42].

H. pylori survive in the acidic environment of the stomach by releasing the enzyme urease. Urease converts urea to ammonia, which suppresses the acidity of the stomach, protecting the bacteria. The reduction in stomach acidity results in hypochlorhydria which may lead to a variety of gut disorders especially digestive disorders. A reduced incidence of infection has been observed in developed countries compared with underdeveloped nations where the carriage rates of infection are as high as 90% however the infection remains prevalent throughout the world [43]. A validated method for the primary isolation and detection of *H. pylori* from dyspeptic patients was developed by Sulami et al. (2008). A positive urease test usually shows a strong indication of *H. pylori* infection. This test is widely used as standard procedure for the detection of this bacterium for being simple, reliable and inexpensive test for an onspot testing [45].

Traditional (Unani medicine) plants from Pakistan are extensively utilized for the cure of gastrointestinal disorders to explore the natural source for pilot compounds against *H. pylori*. Minimum bactericidal concentration (MBC) of the active plants was determined at the concentration range from 7.8 to 500 g/ml evaluated the anti- *H. pylori* activity of 50 commonly. Anti- *H. pylori* activity of medicinal plants has a significant potential source of new bactericidal agents [46].

With the increased use of NSAIDS as analgesics in management of pain, (PUD) Peptic ulcer disease (gastric and duodenal ulcers) is the common gastrointestinal disorder witnessed. An estimated 15,000 deaths occur each year as a consequence of Peptic ulcer disease [47]. NSAIDS commercially such as Indomethacin has been widely prescribed for the management of pain, inflammation and fever in humans. The effects of orally administered *NS* seed oil (ORIGO '100 % natural Black Seed Oil', 2.5 ml/kg; orally) on the gastric tissue in experimental rats revealed a significant increase in gastric tissue catalase (CAT) activity compared with the colitis group indicates that thymoquinone content of black seed exerts superoxide dismutase (SOD) like activity. Thus proving the protective effect of *NSO* on gastric mucosa. The pro-inflammatory cytokines generated in trinitro benzene sulphonic acid (TNBS)-induced colitis affect the gastric tissues samples. The pathogenesis of peptic ulcer disease has largely been investigated recently. The extent of use of numerous newer and better Anti-*H. pylori* drugs are in practice for the treatment of peptic ulcer disease such as Proton pump inhibitors, Histamine receptor blockers, Prostaglandin analogs, which show poor performance and declined rapidly due to highly resistant bacterium of the *H. pylori*. While these drugs showed development of occurrence of relapses and side effects made them arguable. This has been the rationale for the development of new drugs which are effective as analgesic and gastro protective [48]. Seeds of *NS* contain a number of chemical constituents that interact in a complex way to elicit their pharmacological responses. *Helicobacter pylori* is a common bacterium that infects the stomachs of approximately 50% of the world population, while transmission of the bacterium is primarily through compromised water sources but also occurs through person to person transmission [49]. *Helicobacter pylori* is a gram negative curved rod bacterium [50]. Australian physicians Warren and Marshall discovered the unidentified bacteria in the early 1980s in the gastric mucosa and duodenum of patients suffering from peptic ulceration. It is now accepted that *Helicobacter pylori* infection is a precursor to chronic gastritis, peptic and duodenal ulceration [51]. Where gastric inflammation persists for decades, atrophic gastritis can evolve into adenocarcinoma or increase the person's risk of developing mucosa associated lymphoid tissue (MALT) lymphomas [52]. *H. pylori* survive in the acidic environment of the stomach by releasing the enzyme urease. Urease converts urea to ammonia, a strong base, which neutralizes the acidity of the stomach, protecting the bacteria. The reduction in stomach acidity results in hypochlorhydria which may lead to a variety of gut disorders especially

digestive disorders. Ammonia generated by *H. pylori* causes damage to the gastric mucosa inducing gastritis and potentially leading to ulceration [53]. The bacterium takes hold through adhesion host cell interactions between gastric epithelial cells and erythrocytes [54]. Upon infection, rapid reproduction of the bacterium occurs in the stomach causing gastritis and a reduction in gastric acid production. In the majority of people this gastric inflammation associated with *Helicobacter pylori* infection will settle causing asymptomatic gastritis and normal acid production. According to a first clinical research trial in human being suffered from *H. pylori* bacterium showed best results using Black Seed. The amount of 2 g/d of Black Seed resulted in producing a strong impact of (67%) of *N. sativa* on *H. pylori* mortality. While previously in-vitro study represent a 100% hindered the growth of *H. pylori* colonies in media culture [55]. Numerous botanicals were tested *in vitro* in various studies for eradicating *H. pylori* in order to overcome the incidence of rising resistance against antibiotics, while a lot of them express anti-*H. pylori* activity [56].

The alkaloids are natural nitrogen-containing secondary metabolites mostly derived from amino acids and found in about 20% of plants. There has been considerable pharmacological research into the antiulcer activity of these compounds. In this regard, the seeds of *Nigella sativa* L (NS), which is a small annual herb distributed and cultivated all over India, have been reported to have analgesic and gastroprotective effect [57]. Numerous diseases are attributed to *Helicobacter pylori* (*H. pylori*), including persistent active gastritis, peptic ulcer disease and gastric cancer. Antimicrobial agents proved to be effective for the cure of *H. pylori* malady, thus identification of new efficient agents are necessary to research out. *Nigella sativa* seed (NS), is an herb generally possesses *in vitro* anti-helicobacter activity. The efficacy studies for suppression of *H. pylori* bacterium in non-ulcer dyspeptic patients were carried out using NS as a therapeutic agent [58]. Black seed along with its bioactive components such as thymoquinone (TQ) have a shielding effect on the epithelial linings of stomach against the damaging effect induced by pure alcohol and promote curing of ulcer evident from the ulcer index values by inhibiting peroxidative activity, preventing oxidation of free radical and restrict the production of histamine [59]. *Nigella sativa* extract was also proven to have a protective action against ethanol- induced ulcer in rats [60, 61]. Different solvent extracts have different medicinal values such as fixed oil or volatile oil components have antiulcer properties [62]. The effect of alcoholic extract of *Nigella sativa* was

investigated in rats to evaluate the anti-ulcer activity by using two models, i.e. pyloric ligation and aspirin induced gastric ulcer. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, free acidity, total acidity and ulcer index. The results specify that the alcoholic extract significantly ($P < 0.001$) decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control [38].

CONCLUSION

Black Seed (*Nigella sativa*) has significantly fascinated scientists interest during the current era, additionally more research is needed on medicinal aspects of *Nigella sativa*. However evaluation of the health benefits of bioactive components of Black Seed is required necessarily. Likewise, a dynamic area of research evolved based on emerging facts recommend that naturally occurring resources proved to be valuable for identifying new bioactive efficient compounds found in Black Seed.

As the evidence presented in this section will show, it is quite probable that as medical science increasingly learns more about black seed, one or more of its more active ingredients may become combined into a medicinal preblockedion for specific conditions. In the event that this does occur, it is also likely that this particular extract of black seed will be chemically compounded and thus become a more potent medicine. While it may be argued that chemical additives may increase black seed's effectiveness in treating specific conditions, the healing principles of black seed in its pure, natural form should also be taken into account. Black seed, in its complete, natural form, acts on the principle of assisting the body's own natural healing process in overcoming illness or maintaining health. It works on the part or system of the body affected without disturbing its natural balance elsewhere. The body's immune system effected positively and become stable after ingestion of Black seeds. In addition, mostly diseases are resulted due to malfunctioning of immune system. In view of the fact about that the immune system controls directly or indirectly all the functions of body, therefore in the conditions of infection by certain disease or pathogen, the ability of the body's immunity influences the effectiveness of body regarding curing of disease occurred. The use of ethno botanical drugs among Asians as complementary medicine is prevalent and is also gaining increasing popularity in the West. *N. sativa* seeds exert their medicinal effects. With the increased understanding of its mechanism of bioactivity, the incorporation of this medicinal herb as complementary medicine into mainstream medical science can be achieved in the future.

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