Review Article

A REVIEW ON PHARMACOLOGICAL ACTIVITIES OF *NIGELLA SATIVA*

Sravanthi Gandham* and Snehalatha P

Malla Reddy Pharmacy College, Maisammaguda, Dhulapally,

Secunderabad-500014, Telangana, India.

ABSTRACT

Nigella sativa (Black seeds, Kalonji, Kalajira, Black caraway seeds, Black cumin) is an annual herb with many pharmacological properties.*Nigella sativa* seeds have been widely used in traditional medicine as carminative, digestive, antidiarroheal, appetite stimulant, diuretic.Thymoquinone is one of the most active chemical constituent and has wide variety of pharmacological properties.It has many pharmacological activities such as anti-cancer, anti-viral, anti-bacterial, anti-oxidant, anti-parasitic, anti-fungal, anti-diabetic.The present review paper tries to describe the various pharmacological activities that have been carried out by various researchers.

Keywords: *Nigella sativa*, thymoquinone, anti-parasitic, anti-fungal, anti-cancer, anti-diabetic.

INTRODUCTION

Nigella sativa seeds are mostly called as black cumin or black seeds or kalonji or kalajira or black caraway seeds which belongs to the family Ranunculacea. It is an annual herb with many pharmacological activities.It was narrated by Abu hurariah, the prophet Mohammad said "Use this black seeds;it has a cure for every disease except death"[1].The plant is cultivated in India, Bangladesh, Turkey, Mediterranean basin, Middle east mainly for its seeds.The use of N.sativa seeds and oil in traditional remedies goes back more than 2000 years and this herb is described as the Melanthion by Hippocrates and Discroides.N.sativa is characterized by an alternate finely divided ,feathery, grayish, green leaves and an erect branched stem.It grows 20 to 30 cm (7.9-11.8 inch) tall and has linear lanceolate leaves.The fruit of the plant is large and inflated capsule which is composed of 3 to 7 united follicles that each of them has numerous seeds.The black coloured seeds are flattened, angular, oblong, funnel shaped with 0.1 to 0.2 cm wide[2].

CHEMICAL CONSTITUENTS

Extensive studies have been performed to identify the chemical composition of black seeds.The chemical constituents of black seeds include fixed oils(32-40%), proteins(16-19.9%), mineral(1.79-3.74%), fiber(5.5%), water(6%), proteins(16-19.9%)[3]. Fixed oils contain unsaturated fatty acids which include palmitic acid, arachidonic acid, linolenic acid, linoleic acid, stearic acid, myristic acid, oleic acid, beta sitosterol, cycloartenol, sterol esters, sterol glucosides[4]. N.sativa seeds are rich in sitosterol that can inhibit the absorption of dietary cholesterol.Volatile oils contain saturated fatty acids which include nigellone. Nigellone is the only component of the carbonyl fraction of the oil.It also contains thymoquinone, thymol, carvacrol, d-limonene, 4-terpineol, d-citronella, p-cymene, t-anethol, thymohydroquinone, dithymoquinone, longifoline[5] . Black seeds also have carbohydrates which include monosaccharides that are in the form of glucose, mannose, xylose, arabinose. It also have a non starch polysaccharide component that is a valuable source of dietary fibers. Vitamins include thiamine (15µg/g), Riboflavin(1µg/g), folic acid(610 I.U/g), pyridoxin(5µg/g), niacin(57µg/g).Black seeds also contain carotene .Carotene is converted into vitamin A by liver[6]. Black seeds have 15 amino acid.They include 8 of 9 essential amino acids.The essential amino acids in black seeds include lysin, leucine, tyrosine, threonine, isoleucine, methionine, phenylalanine, valine[7].Black seeds include minerals such as copper(18µg/g), iron(105µg/g), phosphorous(5.265mg/g), zinc(60µg/g), calcium(1.89mg/g). Black seeds have two different forms of alkaloids.They are isoquinoline and pyrazole alkaloids.The isoquinoline alkaloids include nigellicimine, nigellicimine-n-oxide.Pyrazole alkaloid include nigellidine and nigellicine[8]. Black seeds include saponins like hedrin, hederagenin.Currently a new acetylated triterpene saponin (penta hydroxyl pentocyclic triterpene) has been isolated .Black seeds also include resins and tannins[9].

TRADITIONAL USES

Nigella sativa seesds are used as a carminative, aromatic, digestive, spice, condiment, diuretic,

diaphoretic, stomachic, liver tonic[10]. It is used for the removal of foul breath and watering from mouth.It is used in obesity and dyspnoea. It is used externally in treatment of alopecia, eczema, freckles, pimples, leucoderma. It can cure obstinate hiccups.It acts as a appetite stimulant.It is used in chronic headache and migraine.Black seeds have anti-bilious property and are administered internally in intermittent fever[11]. Black seeds have bronchodilation action and spasmolytic action.It has calcium antagonist property.It is used as abortifacient in large doses.Constant inhalation of fried black seeds relieves cold and catarrh[12].

PHARMACOLOGICAL ACTIVITIES

Black seeds have anthelmintic[13], anticancer antioxidant[14], antimicrobial[15], antiinflammatory, analgesic, antipyretic[16], antifungal[17], antisporiatic[18], antidiabetic[19].It can be used in treatment of mild to moderate acne vulgaris[20].It is used in treatment of Hepatitis C[21].It has wound healing property[22].It shows its action against CCl_4 induced hepatotoxicity[23] .It treats stomach carcinogenisis.It is used in the therapy of infertility and for inducing the menstruation.It is used in oligomenorrhoea treatment. It is used in uterine disorders related to oxytocin and prostaglandin induced increased contractility[24].It is used in treatment of asthma by inhibiting the release of histamines from mast cells[25].It has post coital contraceptive activity[26].It is a remedy for prophylaxis of cold and asthma.It has immune potentiating characteristics[27].It is used as adjuvant to oral hypoglycemic agent.It is a hypocholesterolemic agent.It has cardioprotective property.It decreases the low density lipoprotein, triglycerides, cholesterol, phospholipids , uric acid levels.It increases high density lipoprotein levels[28].It treats nephrotoxicity induced by cisplatin[29].

ANTI-PARASITIC ACTIVITY

Anti-cestodal effect of *Nigella sativa* was studied in children infected naturally with cestode worms.A single administration of 40mg/kg of ethanolic extract of N.sativa has shown anti-cestodal effect where fecal eggs percent was diminished without any side effects[30].The antimalarial activity of the extract against *Plasmodium yoell nigeriensis* was assessed using Rane test procedure.The methanolic extract of N.sativa extract at a dose of 1.25g/kg body weight significantly ($p<0.05$) suppressed P.yoelli infection in the mice by 94% whereas chloroquin which is the drug of choice in malaria has shown the result about 86%.So the methanolic extract of N.sativa is more effective than chloroquin in the treatment of malaria[31].The anthelmintic activity of essential oil of N.sativa was evaluated against earthworm, tapeworm, hookworm, nodular worm.N.sativa extract has shown the good activity against earthworm and tapeworm.A 400mg/kg of oil emulsion of N.sativa was used to treat coccidiosis in rabbits[32].The N.sativa oil emulsions has higher concentration of alkaloid nigellicine that has a deadly influence on parasites.The essential oil from seeds of N.sativa in pure state and at various dilutions was screened invitro against some microbes and helminths and it was found to exhibit better activity against *Shgella flexnet*[33].

ANTI-VIRAL ACTIVITY

Nigella sativa seeds oil has shown the antiviral activity against Murine cytomegalovirus(MCMV).Intraperitoneal administration of N.sativa seed oil to BALB/c mice, a susceptible strain of MCMV infection has inhibited the virus titers in spleen and liver on day 3 of infection with 10^5 PFU MCMV.On the day 10 of infection the virus titer was undetectable in spleen and liver of N.sativa oil treated mice[34]. *Nigella sativa* seeds has shown anti viral activity against Infectious Laryngotrachietis virus(ILTU) with EC_{50} 35µM[35].N.sativa extract was administered for 3 successive months at a dose of 450mg three times daily in patients with Hepatitis C virus(HCV) who were not eligible for IFN/ ribavirin therapy.N.sativa administration significantly improved the HCV viral load (380808.7$\pm$610937 vs 147028.2$\pm$47522506, p=0.001).N.sativa extract has shown antiviral activity against hepatitis C virus[36].N.sativa seeds has shown the anti viral activity against avian influenza (H9N2) in turkeys.A group of turkeys were fed on diet containing 6% N.sativa seeds extract .The increased cytokine gene expression has shown antiviral behaviour of N.sativa especially in dose dependent manner, leading to suppressed pathogenesis of H9N2 virus[37]. The alcoholic extract of N.sativa seeds(50µg/ml) has shown the antiviral activity against Pestes des petitis ruminants (PPR)[38].N.sativa seeds extract has shown the anti viral activity against broad bean mosaic virus(BBMV). N.sativa extract was more effective in reducing the local lesions produced by BBMV on *Chenopodium amaranticolor* where the percentage of inhibition was found to be 25.71%[39]. N.sativa seeds decoction shown its antiviral activity against Zucchini yellow mosaic virus infecting the *Citrullus lanatus* [[40].*Nigella sativa* seed extract has shown antiviral activity against Human immunodeficiency virus.The ethanolic extract of N.sativa is effective against New castle disease virus(NDV) in terms of

decreased viral load and mortality in embryonated chicken eggs[41].

ANTI-CANCER ACTIVITY

The *Nigella sativa* seeds essential oil nanoemulsion (droplet size is 20 to 50nm diameter) has significantly reduced the viability of Michigan cancer foundation-7(MCF-7) breast cancer cells.The nucleocytoplasmic morphological features of N.sativa essential oil nanoemulsion treated cells included cytoplasmic vacuolation, cell membrane blebbing, marginalization of chromatin and fragmentation of nucleus.Therefore it indicates that the N.sativa essential oil nanoemulsion induced apoptosis in MCF-7 cells[42].The methanolic extract of the seeds of N.sativa exhibited inhibition of cancerous cells growth against HL-60 and U-937 with IC_{50} value 13.70µg/ml, 28.31µg/ml respectively. Invitro experiments were done utilizing non cancerous fibroblasts and a mouse colon carcinoma cells(MC38 cells)[43].Heating the N.sativa seeds to 50^0C, 100^0C, 150^0C produced oil with a strong ability to inhibit tumor cell growth.N.sativa oil from heated seeds delayed the expression of nuclear factor -kappa B transcription.The non heated seeds resulted in 50% inhibition.The IC_{50} for unheated N.sativa seeds was found to be 1.4µg/ml, while the heated N.sativa seeds yielded higher growth inhibition potency towards MC38 cells with an IC_{50} of 0.6µg/ml[44].Thymoquinone of N.sativa has anticancer property.Thymoquinone of N.sativa will upregulate the expression of apoptotic genes (capase-3,8,9 and bax) and down regulate the anti-apoptotic genes(bcl-2) thereby thymoquinone leads to apoptosis and causes cell death.Thymoquinone suppresses Akt activation and causes cancer cell death.Thymoquinone upregulates IL-6, IL-8 production and I Kappa B-alpha degradation which leads to deactivation of NF-Kappa B pathway and thus control the oncogene expression and leads to cancer control.Thymoquinone causes activation of antioxidant enzymes like superoxide dismutase, chloramphenicol acetyltransferase, glutathion peroxidase activities and protects the cell against cancer.Thymoquinone protects the cytochrome P450 enzymes form environmental damage and protects the normal cells from cancer[45]

ANTI-BACTERIAL ACTIVITY

Nigella sativa seeds oil has shown dose dependent antibacterial activity which was more against gram positive bacteria than gram negative bacterial.Among the gram positive bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pyrogenes,* coagulase negative *Staphylococci* were sensitive to the N.sativa oil.*Enterococcus faecalis, Streptococcus agalactiae* were resistant to the N.sativa oil.Among the gram negative bacteria tested, only *Pseudomonas aeruginosa* was sensitive to oil whereas *Acinetobacter baumanni, Citrobacter freundi, Kiebsiella pneumonia, Proteus mirabilis, Proteus vulgaris, Vibrio cholerae* were resistant to the N.sativa oil.The anti-bacterial activity of the N.sativa oil may be attributed to the presence of thymoquinone, thymohydroquinone and thymol in the oil which possessed the anti-bacterial activity[46].*Nigella sativa* seeds has an inhibitory effect on methicillin resistant *Staphylococcus aureus*(MRSA).The strains of MRSA were sensitive to the N.sativa extract at a concentration of 4mg/disc while the extract had an MIC range of 0.2-0.5mg/ml[47].The N.sativa seed oil obtained from solvent extraction and super critical fluid has shown greater anti-bacterial activity than the oil obtained from hydrodistillation and dry steam distillation.N.sativa oil obtained from solvent extraction and super critical fluid has shown its antibacterial activity against *Staphylococcus epidermidis* with MIC $\underline{\geq}$ 4µg/ml whereas the oil obtained from hydrodistillation and dry steam distillation has shown its actibacterial activity against *Staphylococcus epidermidis* with MIC$\underline{\geq}$256µg/ml and 32µg/ml respectively.Thymoquinone exhibited potent growth inhibition activity against gram positive bacteria with MIC ranging from 8 to 64µg/ml[48].N.sativa essential oil has shown its antibacterial activity against gram positive bacteria like *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus pyrogenes and gram negative bacteria like Bacteroides fragilis, Escherichia coli, Pseudomonas aeruginosa. Nigella sativa* seeds oil has shown the weakest antibacterial activity against Pseudomonas aeruginosa (MIC > 1024µg/ml) and strongest antibacterial activity against Staphylococcus epidermidis with MIC ranging from 4 to 256µg/ml.Diethylether extract of N.sativa (25-400µg per disc) has shown concentration dependent inhibition of gram positive and gram negative bacteria.The extract has shown antibacterial synergism with gentamicin, streptomycin and shown additive antibacterial action with spectinomycin, erythromycin, tobramycin, nalidixic acid, chloramphenicol, doxycycline, ampicillin, lincomycin. Diethylether extract of N.sativa can eradicate non fatal subcutaneous staphylococcal infection in mice when injected at the site of infection[49]. A study revealed that

high tannin and high flavanoidal contents in the N.sativa seeds are responsible for the antibacterial activity in the later stages of the germination. Methanolic extracts of N.sativa has shown good inhibitory effects against gram positive and gram negative clinical bacteria strains during germination phases as compared to the seed extract, the extract showed the highest activity from 5th day to 11th day of germination[50].Thymoquinone was tested for its potential to prevent biofilm formation of *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* strains, among them thymoquinone has shown a significant inhibitory effect($P<0.05$) on biofilm formation of *Staphylococcus epidermidis and Staphylococcus aureus* with a dose dependent manner.Thymoquinone induced prevention of 90% of biofilm formation of *Staphylococcus aureus, Staphylococcus epidermidis and Enterococcus faecalis* when used at 75µg/ml, 109µg/ml, 349µg/ml respectively[51]. Thymoquinone at a concentration of 3µg/ml and 6µg/ml was enough to kill gram positive and gram negative bacteria respectively whereas thymohydoxyquinone required 400µg/ml and 800µg/ml to kill gram positive and gram negative bacteria respectively. Thymoquinone and thymohydroxyquinone in combination with antibiotics exerted synergism[52].

ANTI-FUNGAL ACTIVITY

In a study, an intravenous inoculum of *Candida albicans* in a mice has produced colonies of *Candida albicans* in the liver, kidney, spleen of mice.The treatment of mice with 6.6ml/kg of N.sativa seed extract was given once daily for 3 days.Then 24hrs after the inoculation an inhibitory effect on growth of *Candida albicans* in all organs.So N.sativa extract has antifungal activity against *Candida albicans*[53].From the seeds of N.sativa two novel defensins named Ns-D1 and Ns-D2 were isolated and sequenced.This Ns-D1 and Ns-D2 defensins display strong antifungal activity towards a number of phytopathogenic fungi and oomycete-phytophthora infestans[54]. N.sativa seed oil has a strong antifungal activity compared with the conventional fungicide(Flucanazole, Amphotericine B).The $MIC_{50,}$ $MIC_{90,}$ MFC(minimum fungicide concentration) were 2.453, 4.739, 6.360 against *Candida globrata*.The The $MIC_{50,}$ $MIC_{90,}$ MFC were 2.724, 4.939, 6.360 against *Candida krusei.* Mainly Beta-sitosterol, oleic acid, stigmasterol of N.sativa has anti fungal activity[55].Thymoquinone have potent antifungal activity on *Trichophyton mentagrophytes, Microsporum canis and Microsporum gypseum*[56].

ANTI-OXIDANT ACTIVITY

In a study, a rapid evaluation for antioxidants, using two TLC screening methods showed that thymoquinone, t-anethole, 4-terpineol, carvacrol has radical scavenging property.They possessed variable antioxidant activity when tested in Diphenylpicrylhydracyl assay for non specific hydrogen atom or electron donating activity.They were also effective OH radical scavenging agentsin the assay for enzymatic lipid peroxidation in liposomes and deoxyribose degradation assay[57].*Nigella sativa* seed extract has an IC_{50} of 56.88 µg/ml, which is related primarily to the presence of thymoquinone and hydrothymoquinone.The antioxidant activity of essential oil of N.sativa against the DPPH radical has been evaluated by spectrophotometry[58]. The N.sativa oil can be extracted by using supercritical fluid extraction(SFE) and cold press (CP).The antioxidant activity measured by DPPH and IC_{50} was 1.58mg/ml and 2.30mg/ml for supercritical fluid extraction oil and cold press oil respectively[59].The ferric reducing antioxidant power activity for supercritical fluid extraction and cold press oil was found to be 538.67mmol/100ml and 329.00mmol/100ml respectively.This showed that high level of natural antioxidants could be derived from N.sativa oil extracted by supercritical fluid extraction.Almost all the chemical constituents of the N.sativa seeds has shown antioxidant activity except p-cymene.Thymoquinone and ethanolic extracts has shown the best antioxidant activity[60].

ANTI-DIABETIC ACTIVITY

A study was performed to evaluate the effects of Nigella sativa seed crude ethanol extract on insulin secretion in INS832/13 and βTC-let lines of pancreatic β cells and on glucose disposal by C2C12 skeletal muscle cells and 3T3-L-1 adipocytes.An 18 hour treatment with N.sativa amplified glucose stimulated insulin secretion by more than 35% without affecting sensitivity to glucose.N.sativa has also induced β cell proliferation.N.sativa extract has also increased basal glucose uptake by 55% in muscle cells and 400% in fat cells[61].Nigella sativa seed extract can be used in treatment of both type I Diabetics and also type II Diabetics.The administration of N.sativa seed ethanol extract of 2geqplant/kg through intragastric gavage for 4 weeks has showed a progressive normalization of glycaemia.It also increased insulinemia and HDL-Cholesterol.It also decreased liver and muscle triglyceride

content.So N.sativa seed ethanol extract exerts an insulin sensitizing actions by enhancing acetyl-CoA-carboxylase phosphorylation, a major component of insulin independent adenosine monophosphate-activated protein kinase (AMPK) signaling pathway and by enhancing muscle Glut 4 expression[62].Thymoquinone of N.sativa seeds has antidiabetic activity.Thymoquinone can reduce appetite, hepatic gluconeogenesis, glucose absorption in intestine, blood glucose level, cholesterol, triglycerides, body weight and it can stimulate glucose induced secretion of insulin from beta cells in pancreas.N.sativa seed extract improves glucose tolerance as efficiently as metformin.N.sativa has not shown adverse effects and has very low toxicity.In streptozotocin induced diabetic rats, N.sativa seed extract causes gradual regeneration of pancreatic beta cells, increases the lowered serum insulin concentrations and reduces elevated serum glucose[63].

CONCLUSION

The present review reveals the description, active constituents, traditional uses and pharmacological activities of *Nigella sativa*. It also reveals that *Nigella sativa* contains several phytoconstituents including thymoquinone, thymol, carvacrol, d-limonene, 4-terpineol, d-citronella, p-cymene, t-anethol, thymohydroquinone, dithymoquinone, longifoline. It also contains vitamins, carbohydrates, minerals, amino acids, saponins. The plant has been studied for its various pharmacological activities such as antiparasitic, antiviral, anticancer, antibacterial, antifungal, antioxidant, antidiabetic effects. Evidences conclude that *Nigella sativa* seeds have a potential medicinal value and are relatively safe to consume.Because of its miraculous power of healing,it has got the place among the top ranked evidence based herbal medicines. Futher studies and investigations can be performed on the plant for its various pharmacological studies.

REFERENCES

1. Hajra N. Nigella sativa : the miraculous herb. Pak J Biochem Mol Biol 2011; 44 (1): 44-48.
2. Mohammed Abdulrazzaq ASSIa et,al.The Various Effects of Nigella Sativa on Multiple Body Systems in Human and Animals. PJSRR 2016; 2(3): 1-19.
3. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem 1992; 45;239-242.
4. Ramadan MF and Morsel JT. Characterization of phospholipid composition of black cumin (Nigella sativa L.) seed oil. Nahrung 2002; 46:240-244.
5. Nickavar B, Mojab F, Javidnia K and Amoli MA. Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. Z Naturforsch., 2003; 58:9-10.
6. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem 1992; 45:239-242.
7. Al-Gaby,A.M. Amino acid composition and biological effects of supplementing broad bean and corn proteins with Nigella sativa(Black cumin) cake protein, Nahrung 1998;42:290-294.
8. Atta-ur-Rahman, S.Malik. Nigellamine:A new isoquinoline alkaloid from seeds of Nigella sativa. J.Natural Products 1992, 55:676-678.
9. Mehta BK, Mehta P, and Gupta M. A new naturally acetylated saponin from Nigella sativa. Carbohydr 2009; 344: 149-151.
10. B.H.Ali. Pharmacological and toxicological properties of *Nigella sativa,* Phytotherapy research 2003;17:299-30.
11. M.Darwish Sayed. Traditional medicine in health care. Journal of Ethnopharmacology 1980;2; 19-22.
12. Zainab Hadi Kami. Spectacular Black Seeds (Nigella sativa) : Medical Importance Review.Medical Journal of Babylon 2013;10(4):1-9.
13. Mali, R.G. and Mehta, A.A. A Review on Anthelmintic Plants. Natural Product Radiance 2008;7(5):466-475.
14. Kumar et al. Antibacterial, antioxidant, cytotoxicity and qualitative phytochemical evaluation of seed extracts of nigella sativa and its silver np. IJPSR 2019;10(11): 4922-4931.
15. Hanafy MS and Hatem ME. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J. Ethnopharmacol 1991;34(2-3):275-278.
16. Al-Ghamdi, M. S. The anti-inflammatory, analgesic and antipyretic activity of Nigella sativa. Journal of Ethnopharmacology 2001;76(1): 45-48.
17. H. J. Harzallah, E. Noumi, K. Bekir et al. Chemical composition, antibacterial and antifungal properties of Tunisian Nigella sativa fixed oil. African Journal of Microbiology Research 2012;6.(22):4675–4679.
18. L. P. Dwarampudi, D. Palaniswamy, M. Nithyanantham, and P. S. Raghu. Antipsoriatic activity and cytotoxicity of ethanolic extract of Nigella sativa seeds.

Pharmacognosy Magazine 2012;.8(32):268–272.
19. ALAssadi, E.H. and.Al-mzaien K.A. Effect of Glucose Specific lectins, Alkaloid and Volatile Oil Extracted From Nigella Sativa L Seeds in Blood Glucose Level In Normal Rabbits.The Iraqi Journal of Veterinary Medicine 2006;30 (2): 11-21.
20. N. A. Hadi and A. W. Ashor. Nigella sativa oil lotion 20% vs. benzoyl peroxide lotion 5% in the treatment of mild to moderate acne vulgaris. Iraqi Postgraduate Medical Journal 2010; 9(4): 371–376.
21. E. M. F. Barakat, L. M. El Wakeel, and R. S. Hagag. Effects of Nigella sativa on outcome of hepatitis C in Egypt. World Journal of Gastroenterology 2013;19(16): 2529–2536.
22. Yaman, A. S. Durmus, S. Ceribasi, and M. Yaman. Efects of Nigella sativa and silver sulfadiazine on burn wound healing in rats. Veterinarni Medicina 2010;55(12):619-624.
23. Ahmed, Z.A. Protective Effect of Nigella sativa oil against CCl_4-induced Hepatotoxicity in Rats. Al-Mustansiriyah Journal for Pharmaceutical Sciences 2010;8 (2): 46-55.
24. El-Tahir, K. E., Al-Tahir, A. Y. and Ageel, A. M. Pharmacological Studies on Sesame and Nigella sativa Fixed Oils: Effect on the Sensitivities of the Adrenoreceptors, Baroreceptors, Platelets and the Uterus of the Rat. Saudi Pharmaceutical Journal 1999;7(4): 205-215.
25. Chakravarty, N. Inhibition of histamine release from mast cells by Nigellone. Annals of Allergy 1993;70(3): 237-242.
26. Keshri, G., Singh, M. M., Lakshmi, V. and Kamboj, V. P. Post-coital contraceptive efficacy of the seeds of Nigella sativa in rats. Indian Journal of Physiology and Pharmacology 1995;39:59- 62.
27. Haq, A., Abdullatif, M., Lobo, P. I., Khabar, K. S., Sheth, K. V. and Al-Sedairy, S. T. Nigella sativa: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. Immunopharmacology 1995;30(2): 147-155.
28. Al-Salman, H.K.Y. Effect of Volatile Oils of Some Medical Plants on the Blood Glucose and Cholesterol Levels in Mice. Al-anbar journal of agricultural sciences 2008;1 (6) : 284-295.
29. Badary, O. A, Nagi, M. N, Al-Shabanah, O. A, Al-Sawaf, H. A, Al-Sohaibani, M. O. and AlBekairi A. M. Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. Canadian Journal of Physiology and Pharmacology 1997;75(12): 1356-1361.
30. Akhtar MS, Riffat S. Field trial of Saussurea lappa roots against nematodes and Nigella sativa seeds against cestodes in children. J Pak Med Assoc 1991; 41:185-187.
31. Okeola VO, Adaramoye OA, Nneji CM, Falade CO, Farombi EO, Ademowo OG. Antimalarial and antioxidant activities of methanolic extract of Nigella sativa seeds (black cumin) in mice infected with Plasmodium yoelli nigeriensis. Parasitol Res 2011; 108:1507-1512.
32. Baghdadi HB, Al-Mathal EM. Anti-coccidial activity of Nigella sativa L. J Food Agricul Envir 2011; 9: 10–17.
33. Chowdhary A.K.A, A.Islam. Therapeutic potential of volatile oils of Nigella sativa seeds in monkey model with experimental Shigellosis.Phytotherapy Res 1998;12;361-363.
34. Mohamed LabibSalem, Mohammad Sohrab Hossain. Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection. International Journal of Immunopharmacology 2000;22:729-740
35. Dr. Kawther S. Zaher. Observations on the Biological Effects of Black Cumin Seed (Nigella sativa) and Green Tea (Camellia sinensis).Global Veterinaria 2008, 2 (4): 198-204.
36. Eman Mahmoud Fathy Barakat,Lamia Mohamed El Wakeel. Effects of Nigella sativa on outcome of hepatitis C in Egypt.World J Gastroenterol 2013;19(16): 2529–2536.
37. Sajid Umara, Muhammad Tanveer Munirb, Sabir Subhanc, Tariq Azamc,Qamar un Nisac. Protective and antiviral activities of Nigella sativa against avian influenza (H9N2) in turkeys. Journal of the Saudi Society of Agricultural Sciences 2016:1- 7.
38. Kiran Aqil, Muti-ur-Rehman Khan, Asim Aslam, Aqeel Javeed, Rizwan Qayyum. In vitro Antiviral Activity of Nigella sativa against Peste des Petits Ruminants (PPR) Virus. Pakisthan Journal of Zoology 2018;50 (6): 2223-2228.
39. Mohamed.E.F. Inhibition of Broad bean mosaic virus (BBMV) using extracts of Nigella (Nigella sativa L.) and Zizyphus (Zizyphus spina-christi Mill.) plants. The Journal of American Science ,2011;7(12):727-734.
40. Essam.K.F.Elbeshehy. Inhibitor activity of different medicinal plants extracts from Thuja orientalis, Nigella sativa L., Azadirachta indica and Bougainvillea spectabilis against Zucchini

yellow mosaic virus (ZYMV) infecting Citrullus lanatus. Agriculture and Environmental Biotechnology 2017;270-279.
41. Shamim Molla1 , Md. Abul Kalam Azad1. A Review on Antiviral effects of Nigella sativa L. Pharmacologyonline.silae.,2019;2:47-53.
42. Vaiyapuri Subbarayan,Perisamy Jegan Athinarayanan, Ali A.Alshatwi. Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells. Ultrasonics Sonochemistry 2016;31:449-455.
43. Raval B.P, Shah.T.G, Patel.J.D, Patel.B.A, Patel.R.K, Suthar.M.P. Potent anticancer activity of *Nigella sativa* seeds. Archives of Applied Science Research 2010 (2);1;52-56.
44. Riad Agbaria, Adi Gabarin, Arik Dahan, Shimon Ben-Shabat. Anticancer activity of Nigella sativa (black seed) and its relationship with the thermal processing and quinone composition of the seed. Drug Des Devel Ther 2015;9:3119-3124.
45. Md. Asaduzzaman Khan, Han-chun Chen1, Mousumi Tania1 and Dian-zheng Zhang. Anticancer activities of Nigella sativa (black cumin). Afr J Tradit Complement Altern Med 2011;8:226-232.
46. Salman M.T, Khan R.A, Shukla. Antimicrobial activity of *Nigella sativa* Linn. seed oil against multi-drug resistant bacteria from clinical isolates. Indian Journal of Natural Products and Resources 2008;7(1); 10-14.
47. Abdul Hannan, Sidrah Saleem, Saadia Chaudhary, Muhammad Barkaat, Muhammad Usman Arshad. Anti bacterial activity of Nigella sativa against clinical isolates of Methicillin Resistant Staphylococcus Aureus. J Ayub Med Coll Abbottabad 2008;20(3);72-74.
48. L.Kokoska, J.Havlik, I.Valterova, H. Sovova, M. Sajfrtova, I. Jankovska. Comparison of Chemical Composition and Antibacterial Activity of Nigella sativa Seed Essential Oils Obtained by Different Extraction Methods. Journal of Food Protection 2008;71(21):2475–2480.
49. M.S.M.Hanafy, M.E.Hatem. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). Journal of Ethnopharmacology;1991(34) ;275-278.
50. Mohammad Hayatul Islam1 , Iffat Zareen Ahmad1 and Mohammad Tariq Salman. Antibacterial activity of Nigella sativa seed in various germination phases on clinical bacterial strains isolated from human patients. Journal of Biotechnology and Pharmaceutical Research 2012;4(1);8-13.
51. Kamel Chaieb , Bochra Kouidhi , Hanene Jrah, Kacem Mahdouani and Amina Bakhrouf. Antibacterial activity of Thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. BMC Complementary and Alternative Medicine 2011, 11-29.
52. Eman Halawani, Shehar, Taif. Antibacterial Activity of Thymoquinone and Thymohydroquinone of Nigella sativa L. and Their Interaction with Some Antibiotics. Advances in Biological Research 2009, 3 (5-6) 148-152.
53. M. A. U. Khan, M. K. Ashfaq, H. S. Zuberi, A. H. Gilani. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytotherapy research 2007;17(2) 183-186.
54. Eugene A.Rogozhin, Yulia I.Oshchepkova, Tatyiana I.Odintsova, Natalia V.Khadeeva. Novel antifungal defensins from Nigella sativa L. seeds. Plant Physiology and Biochemistry 2011(49)2 131-137.
55. Ali Asdadi1, Hicham Harhar, Saïd Gharby, Zakia Bouzoubaâ, Adil Yadini, Radouane Moutaj. Chemical Composition and Antifungal Activity of Nigella Sativa L. Oil Seed Cultivated In Morocco. International Journal of Pharmaceutical Science Invention 2014;3(11) 9-15.
56. H.Mahmoudvand, A.Sepahvand, S.Jahanbakhsh, B.Ezatpour, S.A.Ayatollahi Mousavi. Evaluation of antifungal activities of the essential oil and various extracts of Nigella sativa and its main component, thymoquinone against pathogenic dermatophyte strains. Journal de Mycologie Médicale 2014; 24(4) 155-161.
57. M. Burits, F. Bucar. Antioxidant activity of *Nigella sativa* essential oil. Phytotheraphy research 2000;14(5) :323-328.
58. Bessedik Amina, Toxicity and anti-oxidant activity of the essential oil of Nigella sativa .Scholars Research Library Der Pharmacia Lettre 2016;8 (15): 245-249.
59. Nameer Khairullah Mohammed, Mohd Yazid Abd Manap, Chin Ping Tan, Belal J. Muhialdin, Amaal M. Alhelli, and Anis Shobirin Meor Hussin. The Effects of Different Extraction Methods on Antioxidant Properties, Chemical Composition, and Thermal Behavior of Black Seed (*Nigella sativa* L.) Oil. Evidence based complementary and alternative medicine;2016:1-10.
60. Goga, A., Hasic, S., Becirovic, S., Cavar, S. Phenolic Compounds and Antioxidant Activity of Extracts of Nigella sativa.Bulletin

of the Chemists and Technologists of Bosnia and Herzegovina.,2012;39:15-19.

61. Ali Benhaddou-Andaloussi,Louis C. Martineau Danielle Spoor. Antidiabetic Activity of Nigella sativa Seed Extract in Cultured Pancreatic β-cells, Skeletal Muscle Cells, and Adipocytes. Pharmaceutical Biology 2008;46(1–2):96–104.
62. Ali Benhaddou-Andaloussi, Louis Martineau, Tri Vuong, Bouchra Meddah.The *In Vivo* Antidiabetic Activity of *Nigella sativa* Is Mediated through Activation of the AMPK Pathway and Increased Muscle Glut4 Content. Evidence based complementary and alternative medicine 2011;1-9.
63. Murli L. Mathur, Jyoti Gaur, Ruchika Sharma, Kripa Ram Haldiya. Antidiabetic Properties of a Spice Plant Nigella sativa. Journal of Endocrinology and metabolis,2011;1(1):1-8.