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Phytochemical Analysis of *Nigella sativa* and it`s Antidiabetic Effect

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Abstract

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines. Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs. According to World Health Organization up to 80% of the people depends on traditional medicinal plants for their medicines. Nature in general, has yielded most common bioactive substances .The plant kingdom contributes in a more meaningful way to supply the useful substances for the treatment of human diseases. Nigella sativa is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds of Nigella sativa used in pickles as spice, have also been traditionally used in treatment of many diseases including diabetes and hypertension. Among many activities exhibited by Nigella sativa and its constituents in animal experiments, antidiabetic property is most important. Nigella sativa Seeds and oil have a long history of folklore usage in various systems of medicines and food. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-e-Nabwi. Extensive studies on Nigella sativa have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties. The present review is focused on its phytochemical analysis and antidiabetic property.

Key Words- Nigella Sativa, Nigellone, Folk Medicine, Herbal Medicine.

INTRODUCTION

Nigella sativa is a spice plant of family Ranunculacea, commonly known as black cumin or black seed. It is an erect herbaceous annual plant. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia, Italy and Afghanistan. In India it is called as Kalonji or kalajeera while in China it is referred as Hak Jung Chou. The seeds of N. sativa are used by the Indian people in pickles as spice and food preservative, while in Egypt these are used as carminative and flavouring agents in bread. Black cumin oil prepared by compressing the seeds of N. sativa is also used for cooking. For centuries, the seeds have been used for medicinal purpose. In old Latin it is called as 'Panacea' meaning 'cure all'. Ayurveda appreciates N. sativa for many qualities and bitter, warming, stimulant nature. In Islamic medicine, the use of the black seeds is recommended in daily use because it is regarded as one of the greatest forms of healing medicine available. Prophet Muhammad once stated that the black seed can heal every disease-except death-as narrated in the following hadith "Hold onto the use of the black seeds for in it is healing for all diseases except death"(Sahih Bukhari vol. 7 book 71 # 592). Traditionally the seeds and its oil are used in several diseases. The seeds are considered as bitter, pungent, aromatic, appetizer, stimulant, diuretic, emmenagogue, galactagogue, anthelmintic, acrid, thermogenic, carminative, anodyne, deodorant, digestive, constipating, sudorific, febrifuge, expectorant, purgative, abortifacient. They are used in ascites, cough, jaundice, hydrophobia, fever, paralysis, conjunctivitis, piles, skin diseases, anorexia, dyspepsia, flatulence, abdominal disorders, diarrohoea, dysentery, intrinsic hemorrhage and amenorrhea. Seed oil is a local anaesthetic[1,2,3]. Nigella sativa has many different chemical ingredients including thymoquinone (TQ) (30- 48%), flavonoids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid. It has been traditionally used for the treatment of different diseases such as respiratory and digestive disorders, kidney and liver dysfunction and rheumatism[4] in different forms[5]. Previous studies have indicated many

medical properties of black seeds, including immunomodulatory activities as well as anti-inflammatory, antimicrobial and antioxidative effects[6]. No toxic effects of NS were observed in animal models[4] and no serious side effects were observed in clinical trials[5]). The present review aim is to assess the effects of Nigella Sativa and Thymoquinone on diabetes.

MORPHALOGY OF NIGELLA SATIVA

According to Ahmad A et al review Nigella sativa an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually coloured white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds[4].

Figure 1 : Showing Nigella sativa Plant and seeds.

SCIENTIFIC CLASSIFICATION OF THE PLANT(7)

Kingdom: Plantae.
Subkingdom: Tracheobionata that is, vascular plant.
Supervision: Spermatophyte.
Order: Ranunculales.
Family: Ranunculaceae-Butter cup family.
Genera: Nigella.
Species: sativa.

Microscopy- Transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangular elongated or nearly columnar, elongated cells. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parencymatous cells and oil globules[8,9].

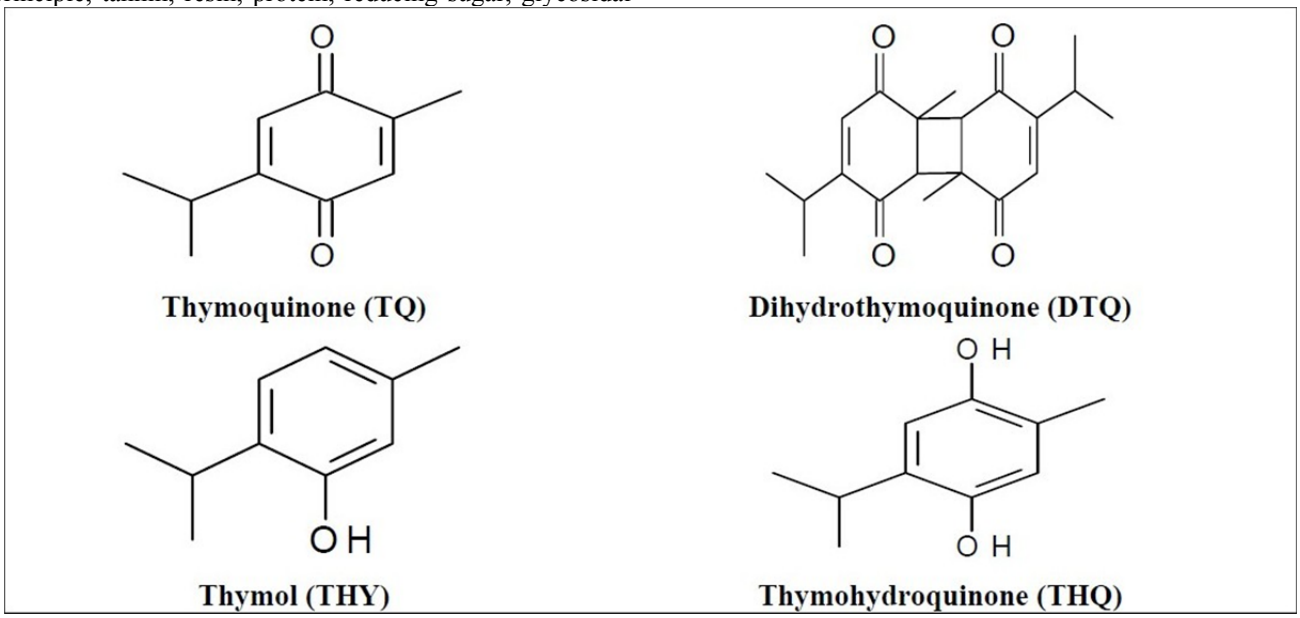
Physical constants

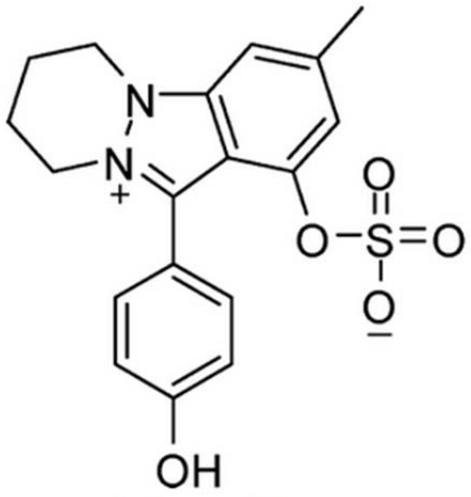
Foreign matter 2% w/w, total ash 6% w/w, acid insoluble ash 0.2% w/w, alcohol soluble extractive 15% w/w, total fixed oil 25-32% w/w, volatile oil 0.42% w/w organic matter, 3.91% w/w loss on drying 4% w/w [10].

Chemical composition of black seeds

According to ahmed aftab et al review many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; that is isoquinoline alkaloids e.g. nigellicimine and nigellicimine- N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Nigella sativa seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent[11,12]. other compounds like carvone, limonene, citronellol were also found in trace amounts. Most of the pharmacological properties of Nigella sativa are mainly attributed to quinine constituents, of which Thymoquinone is the most important. On storage, Thymoquinone yields dithymoquinone and higher oligocondensation products. The seeds of Nigella sativa contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid[11,13]. The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α-sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigmasterol (6.57-20.92% of total sterols)[14,15,16]. In some studies it is reported that the other components includes nigellone,avenasterol-5-ene, avenasterol- 7 - e n e , c a m p e s t e r o l , c h o l e s t e r o l , c i t r o s t a d i e n o l , cycloeucalenol, ,gramisterol, lophenol, obtusifoliol, stigmastanol, stigmasterol-7-ene, β-amyrin, butyro- spermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabino-pyranosyl]-28-O-[α-L-rhamnopyranosyl(1→4)-β-

glucopyranosyl(1→6)-β-D- gluco-pyranosyl] hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol, β-unsaturated hydroxy ketone, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidal saponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamno-pyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16, 23-dihydroxy-28-methy-lolean-12-enoate,stigma-5, 22-dien-3-β-D-gluco-pyranoside, cycloart-23-methyl-7, 20, 22-triene-3β, 25-diol, nigellidine-4-O-sulfite, N. mines A3, A4, A5, C, N. mines A1, A2, B1, and B2[17,18,19,20,21].





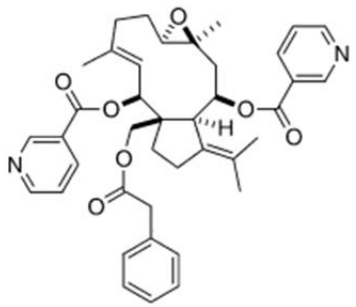


Figure 1 . Showing different chemical components of nigella sativa

STUDIES ON ANTIDIABETIC EFFECT OF NIGELLA SATIVA

Al-Awadi and Gumma[22] have reported the use of a plant mixture containing Nigella sativa, myrrh, gum, asafoetida and aloe by diabetics in Kuwait. They studied the effect of these drugs for their glucose lowering effect in rats and found it to be effective. The Al-Awadi et al.[23], Mohamed et al.[24] studies on the plant mixture containing Nigella sativa revealed that the blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis and the plant extract mixture may prove to be useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus. According to the study of Al-Hader et al[25] the volatile oil of Nigella sativa alone also produced a significant hypoglycemic effect on normal and alloxan induced diabetic rabbits without changes in insulin levels. In study of Farah et al[26] was designed to investigate the possible insulinotropic properties of Nigella sativa oil in streptozotocin plus nicotinamide induced diabetes mellitus in hamsters. After four weeks of treatment with Nigella sativa oil significant decrease in blood glucose level together with significant increase in serum albumin level were observed. The study of Matira et al[27] was confirmed it`s protective effects in diabetes for crude extract and n-Hexane extract of Nigella sativa seed. The

clinical study of Najmi et al[28] demonstrated that significant improvement with reference to total cholesterol, low density lipoprotein cholesterol and fasting blood glucose indicating effective as an add-on therapy in patients of insulin resistance syndrome. Nadia and Taha[29] study evaluated the effect of Nigella sativa seed oil and thymoquinone on oxidative stress and neuropathy in Streptozotocin induced diabetic rats, the results in this study indicated to marked increase in norepinephrine and dopamine concentrations and a marked decrease in serotonin concentration compared to the control group.

The effects of the crude aqueous extract of Nigella sativa seeds (0.1 pg/ml to 100 ng/ml) on intestinal glucose absorption in vitro using a short-circuit current technique and in vivo using an oral glucose tolerance test were investigated. It directly inhibits the electrogenic intestinal absorption of glucose in vitro. Together with the observed improvement of glucose tolerance and body weight in rats after chronic oral administration in vivo, these effects further validate the traditional use of these seeds against diabetes[30]. The possible beneficial effects of Nigella sativa and thymoquinone on histopathological changes of sciatic nerves in streptozotocin induced diabetic rats were evaluated. The treatment of both Nigella sativa and thymoquinone caused a sharp decrease in the elevated serum glucose and an increase in the lowered serum insulin concentrations in streptozotocin induced diabetic rats. Nigella sativa and thymoquinone treatment resulted in increased area of insulin immunoreactive beta-cells significantly. Histological evaluation of the tissues in diabetic animals treated with hymoquinone and especially nigella sativa showed fewer morphologic alterations. Myelin breakdown decreased significantly after treatment with nigella sativa and thymoquinone. The ultra-structural features of axons also showed remarkable improvement suggesting the utility of nigella sativa and thymoquinone as a potential treatment on peripheral neuropathy in streptozotocine induced diabetic rats[31]. Oral administration of ethanol extract of the seeds to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney[32,33].

The possible protective effects of nigella for four week against beta-cell damage from streptozotocin induced diabetes in rats was studied. Nigella sativa treatment has been shown to provide a protective effect by decreasing lipid peroxidation and serum nitrous oxide and increasing antioxidant enzyme activity. Increased intensity of staining for insulin and preservation of beta-cell numbers were apparent in the nigella sativa treated diabetic rats suggesting that nigella sativa treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity[34]. The mechanisms underlying the hypoglycemic effect of nigella sativa oil in streptozotocin induced diabetic hamsters, in terms of hepatic glucose production, and also the possible immunopotentiating effect on peritoneal macrophages were investigated. Treatment with this oil significantly increased the phagocytic activity and phagocytic index of peritoneal macrophages and lymphocyte count in peripheral blood compared with untreated diabetic hamsters suggesting that the hypoglycemic effect is due to, at least in part, a decrease in hepatic gluconeogenesis, and that the immunopotentiating effect of the oil is mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes[35].

The effect of nigella sativa oil, nigellone and thymoquinone were studied on insulin secretion of isolated rat pancreatic islets in the presence of 3, 5.6 or 11.1 mM glucose. It significantly lowered blood glucose concentrations in diabetic rats after 2, 4 and 6 weeks. The blood lowering effect was, however, not paralleled by a stimulation of insulin release in the presence of the oil, nigellone or TQ which suggest that the hypoglycemic effect of oil may be mediated by extra pancreatic actions rather than by stimulated insulin release[36]. The possible protective effects of the volatile oil of nigella sativa seeds on insulin immunoreactivity and ultra 415 structural changes of pancreatic beta-cells in streptozotocine induced diabetic rats were evaluated. Nigella sativa treatment exerts a therapeutic protective effect in diabetes by decreasing morphological changes and preserving pancreatic beta-cell integrity thus suggesting it can be clinically useful for protecting beta-cells against oxidative stress[37]. The plant mixture containing these seed revealed that blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis suggesting its use in non-insulin dependent diabetes mellitus[38].

ANTIOXIDANT EFFECT OF NIGELLA SATIVA

Generation of free radicals may be at least partially the basis of many human diseases and conditions. The effects of nigella sativa oil on the antioxidant enzyme status and myocardium of cyclosporine-A-treated rats was evaluated. Pre-treatment with nigella sativa oil reduced the subsequent cyclosporine A injury in rat heart, demonstrated by normalized cardiac histopathology, decrease in lipid peroxidation, improvement in antioxidant enzyme status and cellular protein oxidation suggesting antioxidant activity as mechanism[39]. The essential oil of Nigella sativa was tested for a possible antioxidant activity by diphenylpicrylhydracyl assay. A rapid evaluation for antioxidants, using two TLC screening methods, showed that thymoquinone and the components carvacrol, anethole and 4-terpineol demonstrated respectable radical scavenging property. They were also effective OH radical scavenging agents in the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay[40]. Thmoquinone was found to exhibit renal protective effect in rats through its antioxidant action[41,42] and also provide protection against hepatotoxicity induced by CCl4 in mice[43], rats and rabbits[44]. The free radical scavenging effects of thymol, Thymoquinone and dithymoquinone were studied on reaction generating reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen using chemiluminescence and spectrophotometric

methods[45]. The hepatoprotective effects of oil and Thymoquinone were found to be via antioxidant mechanism. Similarly the protective effect of Thymoquinone against doxorubicin induced nephrotoxicity[46] and that against doxorubicin induced cardiotoxicity[47,48] were found to be due to antioxidant activity. The modulating effect of thymoquinone on benzopyrene induced cancer in mice[49] and its antitumor effect on 20-methylcholanthrane induced fibrosarcoma tumor genesis were partly due to its antioxidant activity[50]. The antioxidant action of Nigella sativa may explain its claimed usefulness in folk medicine. The essential oil of Nigella sativa was tested for a possible antioxidant activity. The essential oil, thymoquinone and other components like carvacrol, anethole and 4-terpineol demonstrated respectable radical scavenging property. The free radical scavenging effect of thymol, thymoquinone and dithymoquinone were studied on the reactions generating reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen using the chemiluminescence and spectrophotometer methods [51]. Houghton et al[52] reported that Thymoquinone and fixed oil of Nigella sativa inhibit non-enzymatic pero-xidation in ox brain phospholipid liposomes. The antioxidant effect of thymoquinone and a synthetic structurally related ter-butyl thymoquinone were examined in vitro. Badary et al[53] reported that both thymoquinone and ter-butyl thymoquinone efficiently inhibited iron dependant microsomal lipid peroxidation in a concentration dependent manner .

HEPATOPROTECTIVE EFFECT

Hepatotoxicity is associated with alteration in the levels and activities of certain enzymes such as serum glutamic oxaloacetic transaminase- SGO), serum glutamic-pyruvic transaminase -SGPT, oxidant scavenger enzymes system including glutathione -GSH, superoxide dismutase -SOD and catalase -CAT. Daba et al[54] repoted the hepatoprotective effect of thymoquinone against the hepatotoxin: terbutyl hyderoperoxide has been demonstrated using isolated rat hepatocytes . In this study, the hepatoprotective activity of thymoquinone was compared with that of silybin a known hepatoprotective agent. The mechanism of hepatoprotection of thymoquinone is not certain but may be related to the preservation of intracellular gluathione (GSH), the depletion of which by oxidative stress is known to increase the susceptibility of cells to irreversible injury. It has also been shown that pretreatment of rats with Nigella sativa oil for 4 weeks was effective in protection against CCl4 and D-galactosamine induced hepatic damage. No ill effects on liver function were observed when the oil was green orally at a dose of 100 mg/kg/day for 4 weeks. Nagai et al studyi reported in mice thymoquinone, 8 mg/kg/day for 5 days before and 1 day after CCl4 treatment was found to protect against the biochemical and histological markers of liver damage[55]. Fahrettin et al study found to show protective effects against ischemia reperfusion injury on liver[56]. In study of Alcoholic extract of Nigella sativa appears to be a potent candidate to ameliorate the oxidative stress and hepatotoxicity associated with naphthalene in rats and change in some biological markers related to liver disease [57]. In another study confirm the protective role of vitamin E and flavonoids of Nigella sativa seed against hepatic dysfunction caused by sodium nitrate manifested by structural and functional change [58]. In a study confirmed that the black seeds have protective effect of against AlCl3 induced toxicity in rabbits[59]. After reviewing many previous studies we can conclude that the nigella sativa seed blessed with antidiabetic property.

REFERENCES

1. Warrier PK, Nambiar VPK and Ramankutty, Indian Medicinal Plants- A Compendium of 500 species, Vol. 4, Orient Longman Pvt Ltd,Chennai, 2004;139-142.
2. Quality Standards of Indian Medicinal Plants, Ministry ofHealth and Family Welfare, New Delhi, 2005, 161-167.
3. Sharma PC, Yelne MB and Dennis TJ, Database on Medicinal Plants used in Ayurveda, Vol.6, CCRAS, New Delhi, 2005; 420-440.
4. Ahmad, A., Husain, A., Mujeeb, M., Alam Khan, Sh., Najmi, Abul., Siddique, N., et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed 2013; 3(5): 337-352.
5. Paarakh, P. M. Nigella sativa Linn. A comprehensive review. Indian J Nat Prod Resour 2010; 1: 409-29.
6. Tembhurne, S. V., Feroz, S., More, B. H. and Sakarkar, D. M. A review on therapeutic potential of Nigella sativa (kalonji) seeds. Journal of Medicinal Plants Research 2014; 8(3): 167- 177.
7. S. V. Tembhurne, S. Feroz, B. H. More, D. M.Sakarkar. A review on therapeutic potential of Nigella sativa (kalonji) seeds. J. Med. Plants Res..2014 ;8(3):167-177.
8. Khan AA, Ashfaq M and Ali MN, Pharmacognostical studies of selected indigenous plants of Pakistan, Pakistan. Forest Institute, Peshawar, 1979;64-65.
9. Mitra R, Bibliography on Pharmacognosy of Medicinal Plants, NBRI, Lucknow, 1985;362-364.
10. Paarakh, P.M. Nigella sativa Linn.- A comprehensive review. Indian Journal of Natural Products and Resources. 2010;1(4): 409-429.
11. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem 1992; 45: 239-242.
12.]Atta-Ur-Rahman. Nigellidine-a new indazole alkaloid from the seed of Nigella sativa. Tetrahedron Lett 1995; 36(12): 1993-1994.
13. Nickavar B, Mojab F, Javidnia K, Amoli MA. Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. ZmNaturforsch C 2003; 58(9-10): 629-631.
14. Cheikh-Rouhou S, Besbes S, Lognay G, Blecker C, Deroanne C,mAttia H. Sterol composition of black cumin (Nigella sativa L.) and Aleppo pine (Pinus halpensis Mill.) seed oils. J Food Comp Anal 2008; 21(2): 162-168.
15. Mehta BK, Verma M, Gupta MJ. Novel lipid constituents identified in seeds of Nigella sativa Linn. Braz Chem Soc 2008; 19(3): 458-462.
16. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. C R Biol 2008; 331(1): 48-55.
17. Nickavar B, Mojab F, Javidnia K, Amoli MA. Chemic al. composition of the fixed and volatile oils of Nigella sativa L. from Iran. Z Naturforsch C 2003; 58(9-10): 629-631.
18. Morikawa T, Xu F, Ninomiya K, Matsuda H, Yoshikawa M. N.mines A3, A4, A5 and C, new dolabellane-type diterpene alkaloids with lipid metabolism-promoting activities from the Egyptian medicinal food black cumin. Chem Pharm Bull 2004; 52(4): 494-497.
19. Morikawa T, Xu F, Kashima Y, Matsuda H, Ninomiya K, OshikawamM. Noveldolabellane-type diterpene alkaloids with lipid metabolism promoting activities from the seeds of Nigella sativa. Org Lett 2004; 6(6): 869-872.
20. Ali Z, Ferreira D, Carvalho P, Avery MA, Khan IA. Nigellidine-4- O-sulfite, the first sulfated indazole-type alkaloid from the seeds of Nigella sativa. J Nat Prod 2008; 71(6): 1111-1112..
21. Mehta BK, Pandit V, Gupta M. New principles from seeds of Nigella sativa. Nat Prod Res. 2009; 23(2): 138-48.
22. Al-Awadi FM, Gumma KA. Studies on the activity of individual plants of an anti-diabetic plant mixture. Acta. Diabetol. Lat. 1987;24(1):37-41.

23. Al-Awadi FM, Fatania H, Shamte U. The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin-induced diabetic rats. Diab. Res. 1991;18(4):163-168.
24. Mohamed AM, EL-Sharkawy FZ, Ahmed SAA, Aziz WM, Badary OA. Glycemic Control and Therapeutic Effect of Nigella sativa and Curcuma longa on Rats with streptozotocin-induced Diabetic Hepatopathy. J. Pharmacol. Toxicol. 2009;4(2):45-57.
25. Al-Hader A, Aqel M, Hasan Z. Hypoglycemic effect of volatile oil of Nigella sativa seeds. Int. J. Pharmacog. 1993;31(2):96-100.
26. Farah KM, Atoji Y, Shimizu Y, Takewaki T. Insulinotropic properties of Nigella sativa oil in streptozotocin plus nicotinamide diabetic hamsters. Res. Vet. Sci. 2002;73:279-282.
27. Matira K, Zesmin FD. Effects of the crude and the n-hexane extract of Nigella sativa Linn. (kalajira) upon diabetic rats. Bangladesh J. Pharmacol. 2008;4:17-20.
28. Najmi A, Nasiruddin M, Khan RA, Haque SF. Effect of Nigella sativa oil on various clinical and biochemical parameters of insulin resistance syndrome. Int. J. Diab. Dev. Ctries. 2008;28:11-14.
29. Nadia MH, Taha RA. Effects of Nigella sativa Oil and Thymoquinone on Oxidative Stress and Neuropathy in Streptozotocin-Induced Diabetic Rats. Pharmacology 2009;84:127-134.
30. Meddah B, Ducroc R, El-Abbes-Faouzi M, Eto B, Mahraoui L, Benhaddou-Andaloussi A, Martineau LC, Cherrah Y and Haddad PS, Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats, J Ethnopharmacol, 2009, 21(3), 419-424.
31. Kanter M, Effects of Nigella sativa and its major constituent, thymoquinone on sciatic nerves in experimental diabetic neuropathy, Neurochem Res, 2008, 33(1), 87-96.
32. Kaleem M, Kirmani D, Asif M, Ahmed Q and Bano B, Biochemical effects of Nigella sativa L seeds in diabetic rats, Indian J Exp Biol, 2006, 44(9), 745-748.
33. Kanter M, Meral I, Yener Z, Ozbek H and Demir H, Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by Nigella sativa L. in streptozotocin-induced diabetic rats, Tohoku J Exp Med, 2003, 201(4), 213-219.
34. Kanter M, Coskun O, Korkmaz A and Oter S, Effects of Nigella sativa on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats, Anat Rec A Discov Mol Cell Evol Biol, 2004, 279(1), 685-691.
35. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H and Takewaki T, Mechanisms of the hypoglycaemic and immunopotentiating effects of Nigella sativa L. oil in streptozotocin-induced diabetic hamsters, Res Vet Sci, 2004, 77(2), 123-129.
36. El-Dakhakhny M, Mady N, Lembert N and Ammon HP, The hypoglycemic effect of Nigella sativa oil is mediated by extrapancreatic actions, Planta Med, 2002, 68(5), 465-466.
37. Kanter M, Akpolat M and Aktas C, Protective effects of the volatile oil of Nigella sativa seeds on beta-cell damage in streptozotocin-induced diabetic rats: a light and electron microscopic study, J Mol Histol, 2009, 40(5-6), 379-385.
38. Al-awadi F, Fatima H and Shamte U, The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats, Diabetes Res, 1991, 18, 163-168.
39. Ebru U, Burak U, Yusuf S, Reyhan B, Arif K, Faruk TH, Emin M, Aydin K, Atilla II, Semsettin S and Kemal E, Cardioprotective effects of Nigella sativa oil on cyclosporine A-induced cardiotoxicity in rats, Basic Clin Pharmacol Toxicol, 2008, 103(6), 574-580.
40. Burits M and Bucar F, Antioxidant activity of Nigella sativa essential oil, Phytother Res, 2000, 14(5), 323-328.
41. Badary OA, Thymoquinone attenuates ifosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice, J Ethnopharmacol, 1999, 67(2), 135-142.
42. Badary OA, Abdel-Naim AB, Abdel-Wahab MH and Hamada FM, The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats, Toxicology, 2000, 143(3), 219-26.
43. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA and Al-Bekairi AM, Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant
44. mechanism, Biochem Mol Biol Int, 1999, 47(1),153-159.
45. Abuharfeil NM, Salim M and Kleist SV, Augmentation of natural killer cell activity in vivo against tumor cells by wild plants from Jordon, Phytotherapy Res, 2001, 15,109-113.
46. Kruk I, Michalska T,Lichszteld K,Kladna A and Aboul- Enein HY, The effect of thymol and its derivatives on reactions generating reactive oxygen species, Chemosphere, 2000, 41,1059-1064.
47. Al-Shabanah OA, Badary OA, Nagi MN, Al-Gharably NM, Al-Rikabi AC and Al-Bakairi AM, Thymoquinone protects against doxorubicin induced cardiotoxicity without compromising its antitumor activity, J Exp Clin Cancer Res, 1998, 17, 193-198.
48. Nagi M N and Mansour MA, Protective effect of thymoquinone against doxorubicin induced cardiotoxicity in rats: a possible mechanism of protection, Pharmacol Res, 2000, 41, 283-289.
49. Badary OA, Al-Shabanah OA, Nagi MN, Al-Rikabi and Elmazar MM, Inhibition of benzopyrene induced forestomach carcinogenesis in mice by thymoquinone, European J Cancer Prevention, 1999, 8, 435-440.
50. Badary OA and Gamal-el-Din AM, Inhibitory effects of thymoquinone against 20- methylcholanthrene induce fibrosarcoma tumoriogenesis, Cancer Detec Prev, 2001, 25, 362-368.
51. Kruk I, Michalska T, Klanda A. The effect of thymol and its derivatives on reaction generating reactive oxygen species. Chemosphere. 200;41:1059-1064.
52. Houghton PJ, Zarka R, Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995;61:33-36.
53. Badary A, Taha RA, Ayman M, El-Din G, Abdel-Wahab MH. Thymoquinone is a potent suprroxide anion scavenger. Drug Chem. Toxicol. 2003;26(2):87-98.
54. Daba MH, Abdel-Rehman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol. Lett. 1998;95:23-29.
55. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, AL- Bekairy AM. Thymoquinone protects against carbon tetracholide hepatotoxicity in mice via an antioxidant mechanism. Biochem. Mol. Biol. Int.1999; 47:153-159.
56. Farah KM, Atoji Y, Shimizu Y, Takewaki T. Insulinotropic properties of Nigella sativa oil in streptozotocin plus nicotinamide diabetic hamsters. Res. Vet. Sci. 2002;73:279-282.
57. Hamad, Z.M. Protective effect Ethanol extract of Nigella sativa. L on hepatic damage induced by naphthalene in male rats. journal of al-qadisiyah for pure science, 2012;17 (2):1-10
58. AL-Okaily, B.N.; Mohammed, R.S.; Al-Mzain, K.A. and Khudair, K.K. Effect of Flavonoids Extracted from Black Cumin (Nigella sativa) and Vitamin E in Ameliorating Hepatic Damage Induced by Sodium Nitrate in adult male rats. The Iraqi Journal of Veterinary Medicine. 2012;36 (2):172-181
59. Mohammed, A.K. Ameliorative effect of black seed (Nigella sativa L) on the toxicity of aluminum in rabbits. The Iraqi Journal of Veterinary Medicine.2010;34 (2) :110-116.