Chemical Composition of Nigella sativa Linn: Part 2 Recent Advances

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Abstract— This review article is an update on the previous article published on *Nigella sativa* Linn in this journal in 1999. It covers the medicinal properties of *Nigella sativa* and chemical syntheses of the alkaloids isolated from the seeds of the herb.

1.0 INTRODUCTION

The chemical composition and biological properties of *Nigella sativa* L. have previously been reviewed [Khan, 1999; Paarakh, 2010; Ahmad A, et. al., 2013]. In the previous review (Khan, 1999) were reported the large variety of organic compounds that are present in the seeds of *Nigella sativa* L. The seeds of this herb are used in the Middle East and South Asian countries for the treatment of a large variety of ailments and are accepted as a panacea. For example, the seeds or oil from the seeds have been used to control diabetes, hypertension, cancer (leukeamia, liver, lung, kidney, prostate, breast, cervix, skin), inflammation, hepatic disorder, arthritis, kidney disorder, cardiovascular complications and dermatological conditions (Khan *et al.*, 2011; Khan *et al.*, 2003b). A GC-MS analysis of the seed extract has shown it to be a mixture of eight fatty acids and 32 volatile terpenes. The major terpenes, thymoquinone (TQ), dithymoquinone (DTQ), trans-anethol, p-cymene, limonine, and carvone have been identified (Nickavar *et al.*, 2003). TQ and DTQ are both cytotoxic for various types of tumors (Worthen *et al.*, 1998). In addition diterpenes, triterpene and terpene alkaloids have been identified in *Nigella sativa* seeds. The methanolic extract of the seeds contain two types of alkaloids whilst the major principal active ingredient isolated from the volatile oil of *Nigella sativa* L. is TQ. Since *Nigella sativa* L. acts as a panacea exhibiting a wide variety of pharmacological actions discussed previously and updated in this report, interest has arisen in the total synthesis of the alkaloids isolated having the isoquinoline and indazole motifs. The

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isoquinoline alkaloids include nigellicimine (1) and nigellicimine- N-oxide (2), and the indazole alkaloids include nigellidine (3) and nigellicine (4) (Fig. 1). Since the previous review several new dolabellane-type diterpene alkaloids, nigellamines A_1-A_5 (5) have also been isolated from the methanolic extract of the seeds of *Nigella sativa* L. which have also received synthetic interest (Fig. 1). In this update on *Nigella sativa* we want to discuss the chemistry of these various alkaloids and TQ under separate headings.
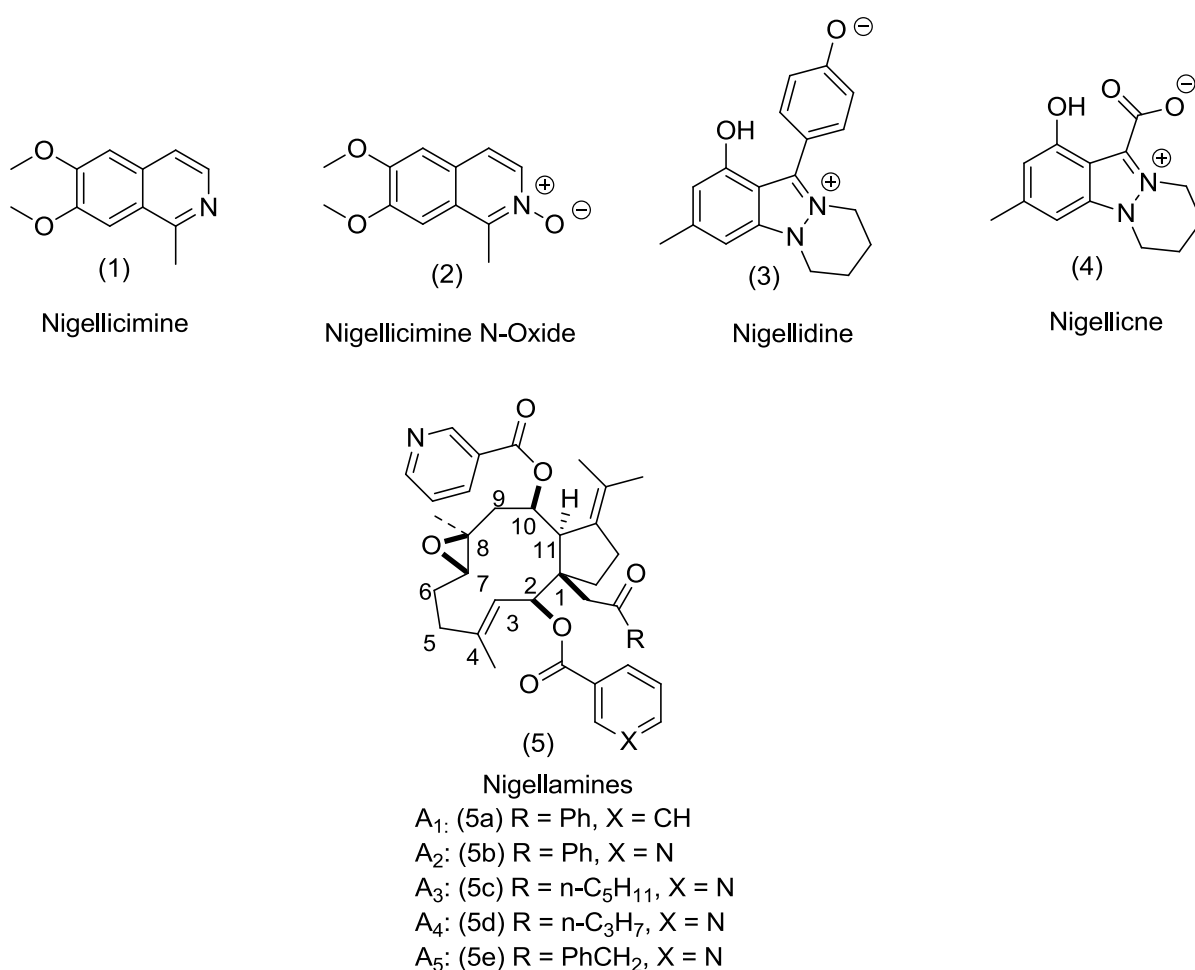

(1)
Nigellicimine

(2)
Nigellicimine N-Oxide

(3)
Nigellidine

(4)
Nigellicne

(5)
Nigellamines
A_1: (5a) R = Ph, X = CH
A_2: (5b) R = Ph, X = N
A_3: (5c) R = n-C_5H_{11}, X = N
A_4: (5d) R = n-C_3H_7, X = N
A_5: (5e) R = PhCH$_2$, X = N

Figure 1. Structures of alkaloids isolated from *Nigella sativa* L.

1.1   Pyrazole and Indazole ring systems

1H-pyrazole   2H-indazole   1H-indazole

Figure 2. Types of indazole ring compounds

Indazole and pyrazole motifs are embedded in numerous pharmaceuticals and agrochemicals with a broad range of biological activities such as (6) (Penning et al, 1997), (7) (Plosker and Goa, 1991), (8) (de Paulis et al, 2006), (9) (Okuno et al, 2004), (10) (Maxwell, 2000) and (11) (Lahm et al, 2009) shown in Figure 3.
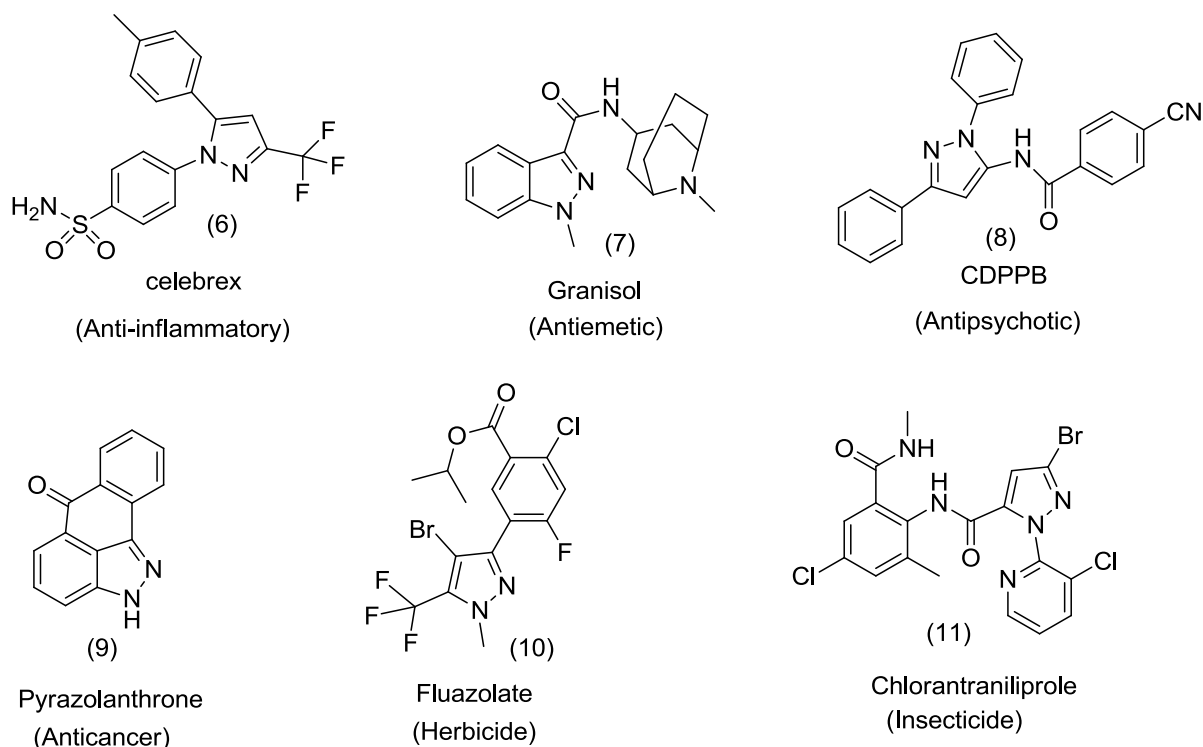

Figure 3. Structures of some pharmaceuticals and agrochemicals with indazole and pyrazole motifs

As a result of these biological activities being associated with the presence of pyrazole and indazole pharmacore in therapeutic compounds, the two indazole alkaloids nigellidine (3) and nigellicine (4) have attracted the attention of synthetic organic chemists for their total syntheses. Thus multigram quantities of these two alkaloids can now be obtained via their total syntheses that should enable their individual therapeutic evaluation to be possible.

2.0 CHEMISTRY OF THE ALKALOIDS AND TQ IN *Nigella sativa*

2.1 Total synthesis of Nigellidine (3)

The development of an efficient synthetic method using Pd(II)/Phen catalyst and conditions for the direct C-3 C-H arylation of (1H) indazole and pyrazole with ArI or ArBr was applied to the synthesis of nigellidine as shown in scheme 1 (Ye et al, 2013). The THP derivative of the commercially available 4-methoxy-6-methyl-(1H)-indazole was reacted with 4-bromoanisole using the C-3 arylation reaction as a key step to form the adduct (14) in 54% isolated yield on the gram scale. Deprotection of the tetrahydropyranyl (THP) group gave (15) which N-alkylation with 1,4-dibromobutane to afford (16) that underwent intramolecular cyclised to furnish the precursor (17). Demethylation of (17) by treatment with BBr$_3$ afforded the natural product nigellidine (3) as the hydrobromide salt in an overall yield of 18%.

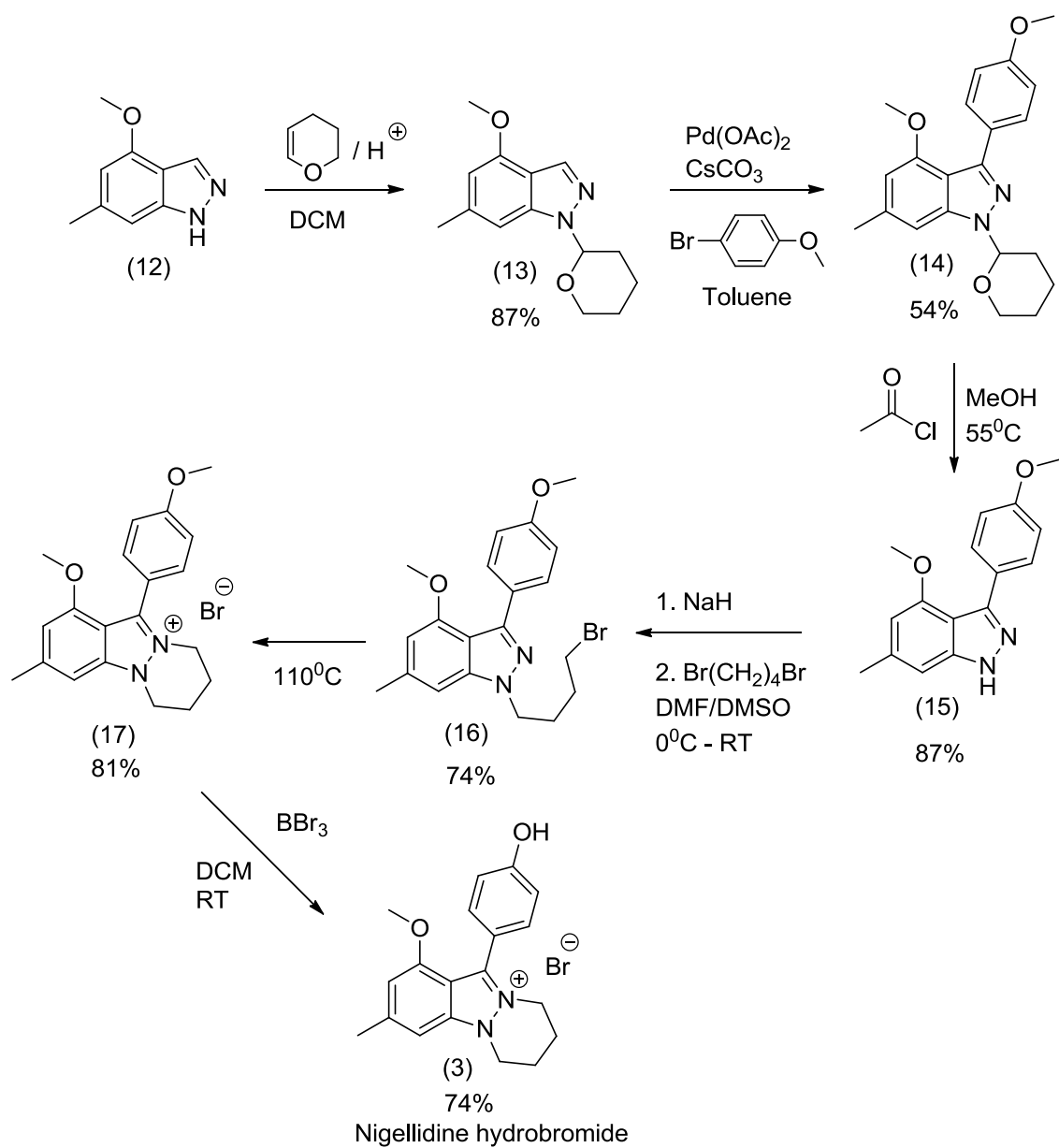### 2.2 The Total synthesese of Nigellicine (4)

To date there have been two total syntheses of nigellicine reported. In the first synthesis shown in scheme 2 commercially available 2-chloro-5-methylphenol (17) was transformed into the protected amide (10) which on lithiation and acylation with diethyloxalate yielded the amide-ester (20) that cyclised on treatment with 6MHCl acid into the isatin (21) (Elliott et al, 2005). Protection of the keto group in isatin (21) as the dimethyl acetal (22) enabled direct amination at nitrogen to give the hydrazine derivative (22) which on treatment with aqueous acid rearranged into an indazole carboxylic acid that was esterified via an acid chloride into (23). Alkylation of indazole ester (23) with 1,4-dibromobutane and subsequent intramolecular cyclisation produced a 4-methoxy derivative of nigellicine which was deprotected with PBr$_3$ to give nigellicine in an overall yield of 18%.

The second total synthesis of nigellicine (4) shown in scheme 3 starts with commercially available 2,5-dimethylphenol (24) which was converted into 2-bromo-6-methoxy-4-methylbenzaldehyde (25) by Clive's method (Inamoto et al, 2007). Treatment of aldehyde (25) with KCN and ethyl chloroformate in the presence of benzyltrimethylammonium chloride (BTAC) and 18-crown-6 in a mixture of water and 1,2-dichloroethane produced an intermediate cyanohydrin carbonate ester which was subsequently converted to a-ketoester (26) by LiHMDS-induced rearrangement. The

reaction of (26) with p-toluenesulfonyl hydrazide gave the key intermediate hydrazone (27) as a mixture of *E*- and *Z*-isomers, which was separable by column chromatography to obtain the major trans isomer that was subsequently converted by Pd-catalysed cyclisation to the indazole (28). Alkylation of the deprotected compound (29) with 1,4-dibromobutane produced the intermediate (30) which underwent intramolecular cyclisation in hot ethanol to furnish the nigellicine ethyl ester hydrobromide salt (31). Finally treatment of ester (31) with PBr_3 caused cleavage of the ester group and deprotection of the methoxy group to give nigellicine (4).

2.3 Total synthesis of Nigellamine A_2 (5b)

The delabellane diterpenes are ubiquitous molecules that are produced by animals, plants, fungi and marine sources and have interesting array of biological activities. The alkaloids nigellamines A_1-A_5 which have been isolated from *Nigella sativa* L. belong to the delabellane family of diterpenes and show potent lipid metabolism-promoting activity (Morikawa et al, 2004a). These biologically active alkaloids have complex structural features and have attracted the attention of synthetic organic chemists for their total synthesis.

Scheme 1. Total synthesis of nigellidine as the hydrobromide salt
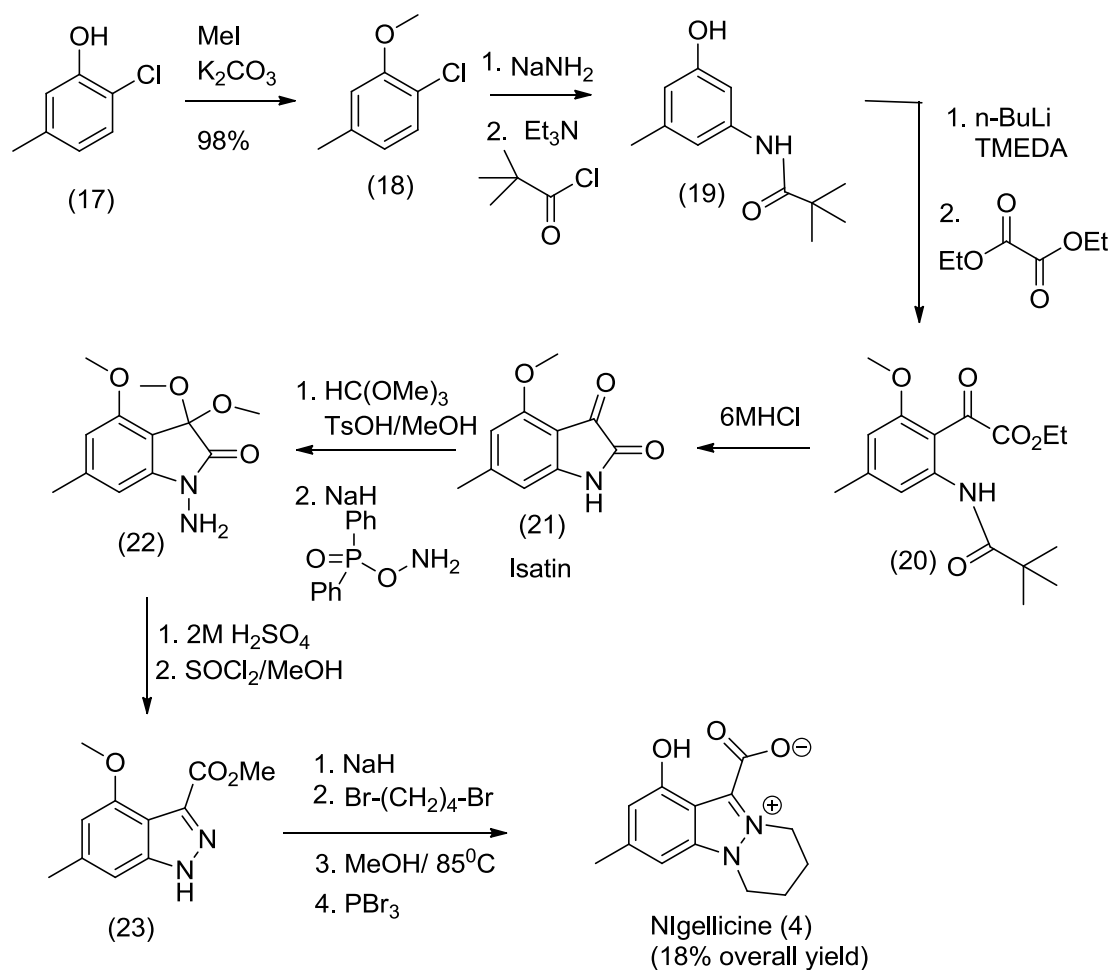
Scheme 2 Total synthesis of nigellicine

One enantioselective total synthesis of nigellamine A$_2$ has so far been reported (Bian et al, 2006). In this synthesis shown in scheme 4 the starting lactone-diene (32) was transformed in three steps and on on multigram scale into the allylic ester (33) as a key intermediate. Iodolactonisation of diene (33) produced (34) which on radical alkynylation furnished the propynyl lactone (35). Desilylation and reduction of (35) yielded the propynyl lactol (36) which upon *in situ* iodination and subsequent silylation afforded the vinyl iodide (37) in good yield. The remaining carbon atoms of the nigellamine skeleton were constructed through cross coupling with alkyl zinc reagent and a repeat methylalumination-iodination sequence of reactions to afford substrate (38). Oxidation with pyridinium chlorochromate (PCC) gave an aldehyde at position C$_2$ which upon sonification underwent Cr-mediated cyclisation with the vinyl iodide group at position
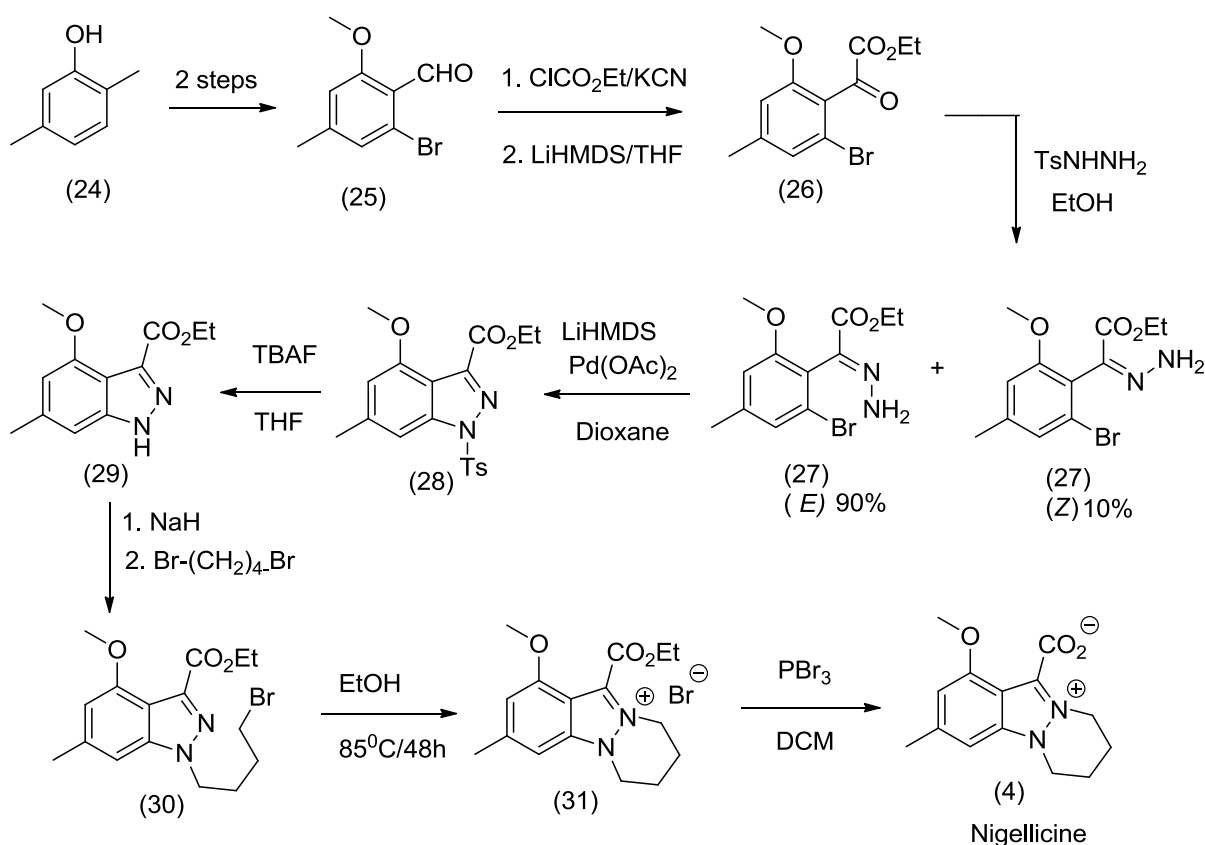
Scheme 3. Second synthesis of Nigellicine (4)

C_3 to generate the 11-membered compound (39). Reductive opening of the lactone and selective acylation of the primary alcohol gave the substrate (40). Oxidation of (40) with Shi's ketone catalyst and oxone proceeded region- and stereoselectively to produce the desired epoxide-diol as the major product which was acylated with nicotinic acid to furnish ent-nigellamine A_2 (5b).

2.4 Novel synthetic Thymoquinone analogues

The compound 5-isopropyl-2-methyl-1,4-benzoquinone is known as thymoquinone (TQ) (41) shown in scheme 5. TQ is the major active principle of the oil of *Nigella sativa* L. and has been shown to exhibit anti-tumor activity against breast, lung, prostrate, liver, colon and pancreatic cancer. Thus interest has arisen to synthesise more potent analogues of TQ. Recently reported are the novel analogues of TQ consisting of compounds (44a-b) were synthesised in two steps from TQ Sodium azide added to TQ in acetic acid to afford the reduced product (42) which on reaction with the aldehydes

(43a-b) generated the Schiff bases (44a-b) (Yusufi et al, 2013). These analogues have shown superior proliferative activity, excellent chemo-sensitizing activity against pancreatic cancer *in vitro* and in combination with Gemcitabine.
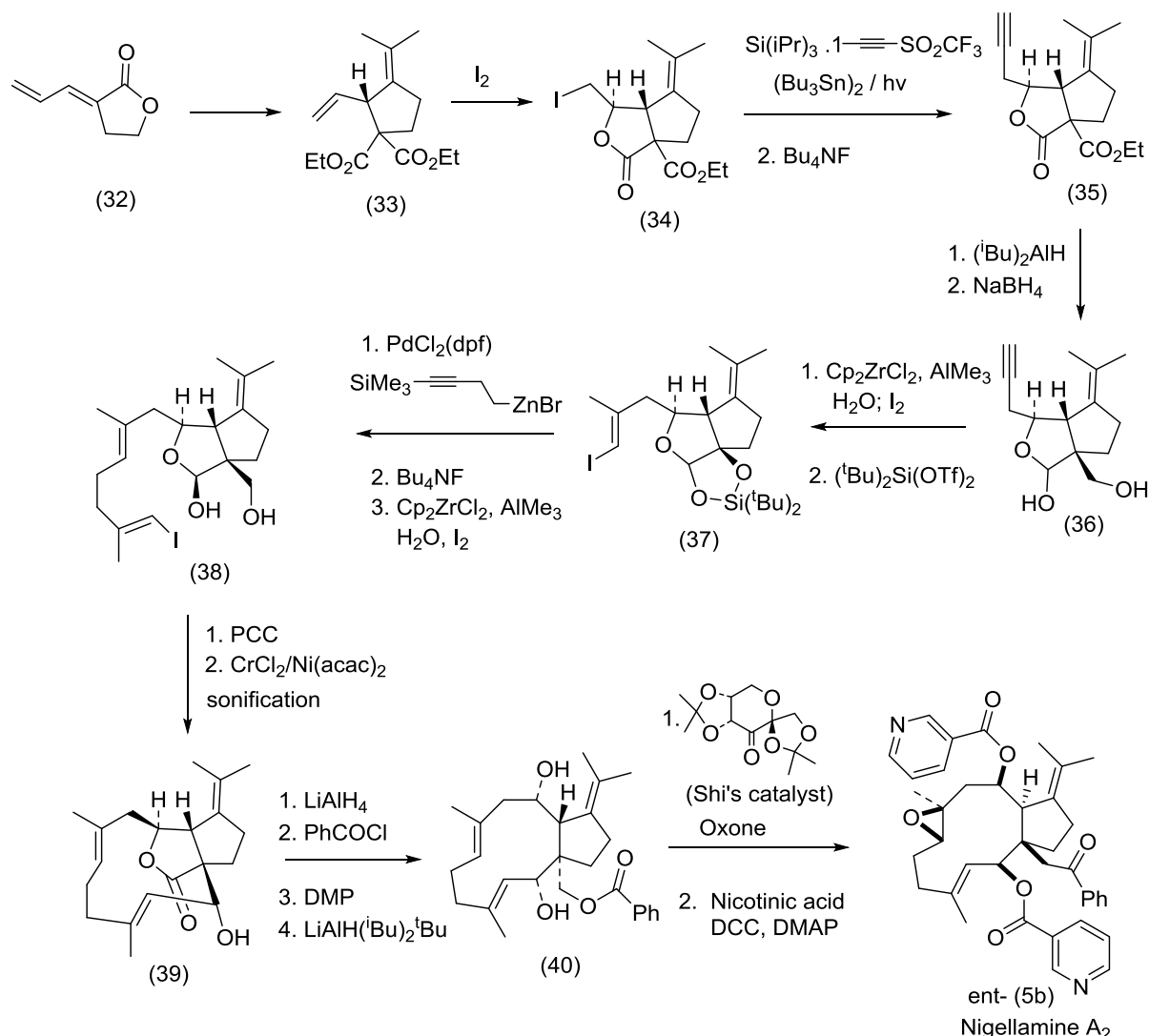
Scheme 4. Total synthesis of nigellamine A_2

One serious drawback with TQ is its toxicity at high doses and poor water solubility which limit its usage as a therapeutic agent. In order to alleviate this problem various types of nanocarrier for thymoquinone have been synthesised (Ravindran et al, 2010; Ganea et al, 2010; Alam et al, 2012; Singh et al, 2013). One recent study has reported the synthesis of PAG coated NIPAAM nanoparticles that are encapsulated with TQ for direct hepato-targeting. NIPAAM is a thermosensitive nanopolymer which is widely used as a successful drug delivery system against various diseases and PAG is a galactosylated moiety that targets the liver by interacting with asialoglycoprotein receptor (ASGP-R)

present on the surface of hepatocytes and delivers the drug directly to the liver (Verma et al, 2013). The toxicity of the nanocarrier (NIPAAM) at this concentration is almost negligible and due to the size of the nanoparticle being smaller than the already reported nanothymoquinone.
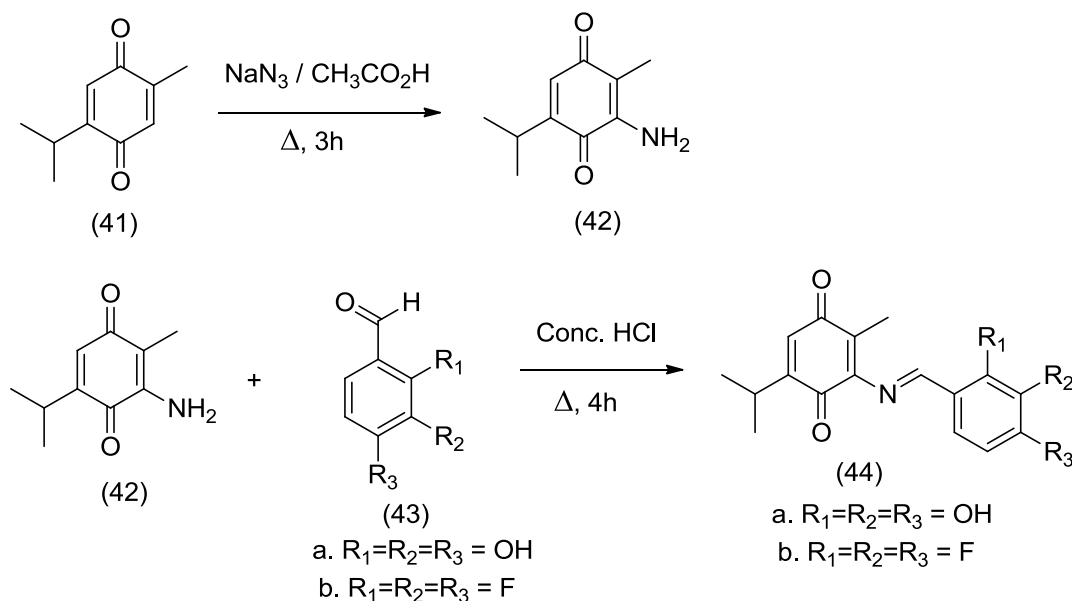
Scheme 5. Synthesis of Thymoquinone alanogues as anticancer agents

This study clearly has demonstrated that the nanoparticles are able to carry bulk amounts of drug to the liver, and their direct targeting to ASGP-R receptors present on hepatocytes has resulted in significant hepatoprotection at a low dose level that is 1000 times lower than the naked TQ. This nanocarrier approach offers a promising prospect for the future against various liver diseases.

3.0   BIOLOGICAL ACTIVITIES OF *Nigella sativa*

3.1   The anti-inflammatory activities

In animal studies *Nigella sativa* shows dose-dependent suppression of nociceptive pain response and cestocidal activity. These activities are shown by TQ that acts through indirect activation of the supraspinal mu(1)- and kappa-opioid receptor subtypes (Abdel-Fattah *et al.*, 2000; Akhtar *et al.*, 1991). The antihypertensive principal TQ and other constituents of *Nigella sativa* are also protective agents against the chromosomal aberrations induced by schistosomiasis (Aboul-Ela, 2002; el el Tahir *et al.*, 1993a).

These compounds are used in the control of arterial blood pressure, anticholinergic, antihistaminic, tracheal relaxation, control of asthma and in the treatment of other allergic diseases (Ahmed *et al.*, 2013; Al-Majed *et al.*, 2001; Boskabady *et al.*, 2004; Kalus *et al.*, 2003; Steinmann *et al.*, 1997).
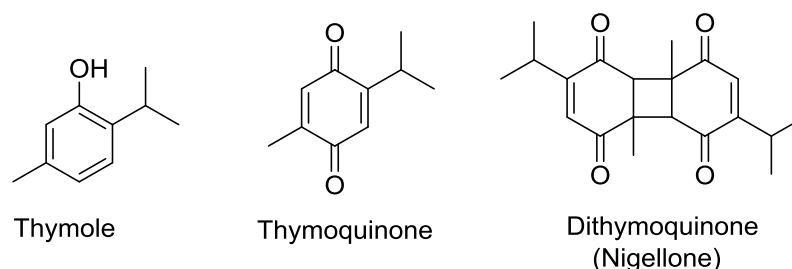

Figure 4. Chemical structures of principal active ingredient isolated from the volatile oil of *Nigella sativa* L

Nigellone (dithymoquinone) is the carbonyl dimer of TQ present in *Nigella sativa* and it inhibits the release of histamine giving relief in asthmatic conditions (Chakravarty, 1993; el Tahir *et al.*, 1993b). The spasmolytic and bronchodilator activities of *Nigella sativa* are mediated possibly through calcium channel blockade (Gilani et al., 2001). Physologically important activities shown by *Nigella sativa* include analgesic, anti-inflammatory, antimicrobial, antifungal and antibacterial effects (Hanafy *et al.*, 1991; Khan *et al.*, 2003a; Morsi, 2000) and CNS activity of its aqueous extract and volatile oil components (Al-Ghamdi, 2001; Al-Naggar *et al.*, 2003; Hajhashemi *et al.*, 2004; Haq *et al.*, 1995). The neuroprotective activity of *Nigella sativa* on neurotransmitter leading to antiepileptic activity has also been described (Arafa *et al.*, 2013). TQ, through an opioid receptor-mediated, increases in GABAergic tone, exhibits anticonvulsant activity in the petit mal epilepsy (Hosseinzadeh *et al.*, 2004).

3.2   Antiulcer and anticancer properties

Ethanol induced ulcer in rats has been reduced by *Nigella sativa* extracts (El-Dakhakhny *et al.*, 2000a). Ischaemia/reperfusion are linked by free radical generation and this could be controlled by an administration of TQ which could offer gastroprotective effects against gastric lesions (El-Abhar *et al.*, 2003). The chemosensitising effect of TQ

in the treatment of 5-Fluorouracil induced gastric cancer has been reported (Lei *et al.*, 2012).

3.3   Hepato-protective antioxidant activities

The aqueous extract of Nigella sativa (NS) is hepato-protective against carbon tetrachloride induced oxidative hepatic damage suggesting powerful antioxidative properties of NS extract (Al-Ghamdi, 2003; el-Dakhakhny *et al.*, 2000a; Mansour *et al.*, 2001; Meral *et al.*, 2003). NS protects liver by inhibiting enzyme leakage from hepatocytes caused by toxic substances such as carbon tetrachloride (Kanter *et al.*, 2003a). Through its antioxidant action, TQ is known to inhibit 5-lipoxigenase and 5-hydroxy-eicosatetraenoic acid (5-HETE) products suggesting its use in inflammatory pathogenesis (El-Dakhakhny *et al.*, 2002a). Hyperhomocysteinemia (HHcy) has been linked with oxidative stress. Therefore, NS has been demonstrated to improve total antioxidant status in rats treated with methionine induced HHcy (El-Saleh *et al.*, 2004). The oxygen free radical generated by gentamicin pathogenesis causing hepatotoxicity and nephrotoxicity are quenched by oil and seeds of NS (Ali, 2004) and the ethanolic extracts of NS have the potential to protect against gama-radiation induced oxidative damage (Rastogi *et al.*, 2010). It has been reported that TQ inhibits the leakage of hepatic enzymes and the intracellular depletion of GSH protecting liver (Daba *et al.*, 1998).

3.4   Immunomodulatory effect

NS has established immunosuppressive and cytotoxic properties (Islam *et al.*, 2004) and the pharmacological and therapeutical properties of NS have been reviewed by many workers (Ahmad et al., 2013; Ali *et al.*, 2003; Swamy *et al.*, 2000). The splenocyte proliferation, macrophage function, and NK anti-tumor activity of NS have revealed the potent immunomodulatory properties of the Nigella seeds (Majdalawieh *et al.*, 2010). The NS seed oil also shows hepatoprotective action against hypervitaminosis A and humoral immune responses and non-specific cellular immune responses (Al-Suhaimi, 2012; Al-shatwi, 2014). The immune modulating effect of NS is mediated through direct

stimulation of macrophage phagocytic activity or lymphocytes activation (Fararh *et al.*, 2004; Haq *et al.*, 1999).

NS is a known immune stimulant that protects against many pathological conditions (Corder *et al.*, 2003; Fararh *et al.*, 2004). Thus, the powerful antioxidative and protective properties of TQ in proteinuria and hyperlipidemia associated with nephrotic syndrome have been evaluated (Badary et al., 2000). Along with TQ and other terpenoid compounds such as carvacrol, trans-anethole and 4-terpineol with antioxidant properties have been reported for NS (Burits *et al.*, 2000). Carvacrol is a known inhibitor of human neutrophil elastase and may be useful agent in phytotherapy for injuries such as chronic obstructive pulmonary disease and emphysema (Kacem *et al.*, 2006). The volatile NS oil shows anti-oxytocic and sperm characteristics due to its antioxidant activities (Mansour *et al.*, 2013; Aqel *et al.*, 1996).

## 3.5   Effect on Blood sugar and lipid profile

Streptozotocin (STZ) treated animals respond to NS extracts with normalizing blood glucose through extrapancreatic actions rather than by stimulated insulin release and ascertain to be protective against type-2 diabetes (El-Dakhakhny *et al.*, 2002b; Fararh *et al.*, 2004; Fararh *et al.*, 2002; Hawsawi *et al.*, 2001). The significant increase in lipid peroxidation by STZ is also controlled by NS and has protective effect in diabetes by decreasing oxidative stress and regeneration/proliferation of the beta-cells in the islets of Langerhans (Kanter *et al.*, 2004; Kanter *et al.*, 2003b). A petroleum ether extract of NS exhibits insulin-sensitizing activity (Le *et al.*, 2004) and the mechanism of NS extract in the control of diabetes has ben shown to be through controlled insulin release (Rchid *et al.*, 2004). At the same time, amendment in the blood lipids profile has been suggested by the use of NS extracts (El-Dakhakhny *et al.*, 2000a). Arachidonic acid induced blood platelet aggregation and blood coagulation are inhibited by NS indicating its potential use in thrombosis (Enomoto *et al.*, 2001). TQ is involved in the inhibition of arachidonic acid generated eicosanoids and lipid peroxidation (Houghton *et al.*, 1995).

3.6 Effect on arthritis

In human, TQ has been shown to be effective in rheumatoid arthritis (Gheita *et al.*, 2012). Inhibition of arachidonic acid generated eicosanoids (thromboxane B2, leukotriene B4) supports the use of NS for the treatment of rheumatoid arthritis and other inflammatory diseases (Houghton *et al.*, 1995). TQ has been implicated in bone healing in an animal model (Kirui *et al.*, 2004). Inhibition of leukotrienes through 5-lipoxygenase and LTC4 synthase activities in eicosanoid pathway has been well documented (Mansour *et al.*, 2004).

3.7 Anticancer Activity of TQ

A number of antitumor compounds have been identified from NS. These compounds are TQ, alpha-hederin a triterpene, isopropylmethylphenols and dollabelane-type diterpene alkaloid nigellamine A3, A4, A5 and C (Kumara *et al.*, 2001; Michelitsch *et al.*, 2004; Morikawa *et al.*, 2004a; Morikawa *et al.*, 2004b). Thus, numerous types of cancers such as Ehrlich ascites carcinoma (EAC), Dalton's lymphonia ascites (DLA) and Sarcoma-180 (S-180) cells, colon carcinoma, pancreatic carcinoma and hepatic carcinoma have been treated with NS extracts *in vitro* (Salomi *et al.*, 1992; Samarakoon *et al.*, 2010). Changes in intracellular GSH and redox status for mitochondrial function are important factors in the mechanism of alpha-hederin induced cell death (Swamy *et al.*, 2003).

a-hederin
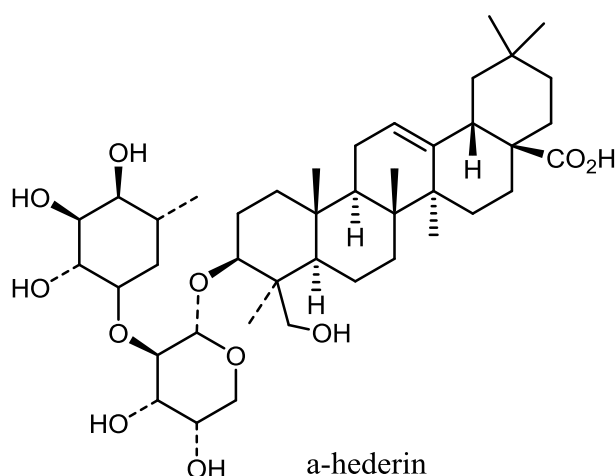
The NS extract exerts anti-hepatocarcinoma effect through modulation of apoptosis (Samarakoon *et al.*, 2012). The regulation of pro- and anti apoptic genes by NS has been demonstrated in treating cervical cancer (Shafi *et al.*, 2009). In many cases the antitumor activity of NS seeds has been attributed to the volatile component thymoquinone (structurally related to tert-butylhydroquinone, a potent antioxidant) that has the potential to protect rat liver against diethynitrosamine (DEN) induced hepatocarcinogenesis (Iddamaldeniya *et al.*, 2003). It also improves the therapeutic efficacy of ifosfamide by decreasing nephrotoxicity and improving antitumor activity (Badary, 1999; Saleem *et al.*, 2012).

TQ also affects the benzo-a-pyrene induced clastogenic activity in rats and 20-methylcycloanthrene induced fibrocarcinoma is inhibited by TQ present in NS extracts (Badary *et al.*, 2007; Badary *et al.*, 2001). While supplementation by NS and honey in the treatment of methylnitrosourea induced inflammation, carcinogenesis and oxidative stress has been reported (Mabrouk *et al.*, 2002), the lipid peroxidation induced liver damage in diabetic rats has also been mentioned (Meral *et al.*, 2001).

The pro-oxidant nitric oxide production is inhibited by NS extracts validating the fact that NS has anti-inflammatory activities (Mahmood *et al.*, 2003). Model i*n vivo* experiments with Schistosomiasis mansoni infected mice have concluded that NS extract have a great protective potential against oxidative stress protecting liver (Mahmoud *et al.*, 2002). The mode of action of TQ against cancer has been suggested to be through its antioxidative properties and interaction with DNA synthesis. The antioxidant and pro-oxidant properties of TQ have been substantiated by augmented TQ mediated scavenging of superoxide anion (Badary *et al.*, 2003). However, presence of the phenolic compounds in NS, such as vanillic acid, could also contribute to the antioxidant properties of NS. These compounds may also be responsible for its antimutagenic activities (Bourgou *et al.*, 2008; Khader *et al.*, 2010). TQ exhibits advanced antimyeloma activity in MDN and XD2 multiple myeloma malignant plasma cells (Badr et al., 2011). However, the mechanism of chemotaxis of malignant plasma cells is not well defined.

3.8 Effect of TQ on Pancreatic carcinoma (PC)

PC is one of the most deadly cancers with almost invariably fatal consequences. TQ has antitumor activity against PC. To combat PC, the dose of TQ has to be high. Therefore, many attempts have been made to study structure activity relationships by synthesizing TQ analogs and some of these compounds have potent antitumor activity against PC (Banerjee et al., 2010). Gemcitabine- or oxaliplatin-induced activation of NF-kappaB is inhibited by TQ, resulting in the chemosensitization of pancreatic tumors to conventional therapeutics (Banerjee *et al.*, 2009). Progressive apoptosis is also inhibited by NS (Corder *et al.*, 2003).

3.9 Co-administration of NS with other substances

Cisplatin is a widely used drug that induces kidney toxicity. It has been established that when cisplatin is co-administered with NS, the nephrotoxicity is reduced (El-Daly, 1998; Nair *et al.*, 1991; Ulu *et al.*, 2012). A co-administration of NS with green tea extract prevents cytotoxicity of organophosphorus compounds (Korany *et al.*, 2011). Co-administration of saffron with NS in the treatment of chemical carcinogenesis has also been reported (Salomi *et al.*, 1991).

4.0 Breast Cancer

It is one of the most common causes of death in women and there is no effective treatment except mastectomy. Many substances have been shown to have mammary anticancer activity and among these are melotonin and retinoic acid. NS has been examined in animals exposed to 7,12-di-methylbenz(a)anthracene (DMBA), mammary cancer causing substance which showed NS reduces the carcinogenic effects of DMBA (El-Aziz *et al.*, 2005). Inactivation of MCF-7 breast cancer cells has been demonstrated by NS extracts (Farah *et al.*, 2003).

4.1   Colon Cancer

The molecular mechanism of action of TQ in colon cancer has been suggested. Thus, colon cancer is inhibited in G1 phase cell cycle and apoptosis is mediated by TQ (Gali-Muhtasib *et al.*, 2004). The 1,2-dimethylhydrazine (DMH), colon cancer inducer, damage erythrocytes has been reported and NS has the ability to detoxify DMH (Harzallah *et al.*, 2012; Worthen *et al.*, 1998). The preneoplastic lesions for colon cancer have been investigated and found that colon cancer in post-initiation stage can be prevented by volatile components of Nigella seeds (Salim *et al.*, 2003).

5.0   Conclusion

The Islamic claim made by prophet Muhammad over 1400 years ago that "black seed (*Nigella sativ*a Linn.) has the cure for all deseases" has a much more meaningful and acceptable dimension to it given the overwhelming scientific data obtained, as outlined in the reviews, that supports it. The alkaloids present in *Nigella sativa* Linn. could now be obtained by total synthesis and the study of their pharmacological properties should make very interesting research studies for the future.

6.0   Acknowledgements

7.0   References

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