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Nigella sativa (NS) seeds contain more than 30 % fixed oil, 0.20-0.45 % (wt/wt.) of essential oil (EO), also known as volatile oil (VO), 1-4 % methanol extract (ME) or nonsaponifiable fraction (NSF) and the rest moisture, protein, ash, carbohydrate and minerals (Ali and Blunden, 2003; kandil 2002; Kanter et al., 2006). The EO is a pale yellow liquid with a characteristic aromatic odour which is readily soluble in ether, methanol, ethanol and chloroform but sparingly soluble in water (El-Alfey et al., 1975). Thymoquinone (TQ) is the major bioactive component in Nigella sativa EO extract (Arslan et al., 2005). Methanol extract (NSF) isolated from NS seed powder contains TQ rich EO and total sterols including phytosterols (Kandil, 2002). Phytosterols are of great interest due to their strong health beneficial antioxidant activity (Ramadan and Morsel, 2004). This fraction with a complex composition constitutes an average 0.3-2 % of the oil but it can reach more than 10 % in certain plants (Stuchlik and Zak, 2002). Our results show an average ME (NSF) percent yield of 1.252 ± 0.002 from 41 extractions of NS seed powder with methanol, while the average percent yield of EO extract from 34 hydrodistillations of NS seed powder was 0.166 ± 0.008 %. The GC-MS analysis of 18 VO samples led to the identification of 32-46 components (Burits and Bucar, 2000; Nickavar et al., 2003; Benkaci-Ali et al., 2006). The major components of each VO samples consisted of a mixture of compounds with low molecular weight monoterpenes, representing about 16-86 % of the total VO content (Burits and Bucar, 2000; Nickavar et al., 2003; Arslan et al., 2005; Benkaci-Ali et al., 2006). The main compounds were TQ (8-57 %), limonene (LMN) (0.29-4.30 %), p-cymene (7-15 %), carvacrol (0.32-11.60 %), 4-terpineol (0.30-6.59 %), t-anethole (0.25-38.30 %), and sesquiterpene longifolene (0.70-8.00 %) (Burits and Bucar, 2000; Nickavar et al., 2003; Benkaci-Ali et al., 2006).

Lipid peroxidation is a critical problem affecting food quality and stability. Two factors promote the development of novel natural antioxidants for food applications. The first factor is consumer preference of natural antioxidants due to the reported carcinogenic activity of synthetic antioxidants, including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Onyeneho and Hettiarachchy, 1992). The other factor is related to the so-called “polar paradox” (Frankel et al., 1996a), which describes the observation that polar antioxidants are
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more effective in nonpolar lipids, while nonpolar antioxidants are more active in polar lipid emulsions (Frankel et al., 1996a). Therefore, food rich in bioactive factors has become an important approach for more consumers, to achieve their desires to reduce the risk of a specific disease or a health problem, and to treat minor illnesses (Sloan, 2000). Development and characterization of the bioactive factors in novel food and agricultural products are required to provide scientific evidence for improving quality and nutritional value of the human diet. These are also important for improved utilization of food and agricultural products. Diphenylpicrylhydrazyl (DPPH) is a stable radical, and has been used to estimate the radical-scavenging capacities of antioxidants and to evaluate the kinetics and thermodynamic properties of radical-antioxidant reactions (Yu et al., 2002a). Results depicted in Fig. 3.1 represent the comparison of DPPH• scavenging activities of ME and EO fraction of NS seed powder with pure TQ and pure LMN, two constituents of EO, used in the present study. These fractions/compounds directly reacted with and quenched DPPH radicals in a concentration dependent manner. The DPPH• scavenging capacities of the above test fractions/compounds were in the order ME > pure TQ > EO extract > pure LMN. Previous reports have shown that both NS seeds (Burits and Bucar, 2000) and ME from cold-pressed black caraway (NS) seeds (Yu et al., 2005) contained radical-scavenging agents that could directly react with and quench stable DPPH•. It is noteworthy that among the four test fractions/compounds, ME had the strongest DPPH• scavenging activity with an IC₅₀ value of 40 µg/ml, followed by 58 and 86 µg/ml for pure TQ and EO extract, while an IC₅₀ value of 8.5 mg/ml was obtained for pure LMN. These results established that ME of NS seeds exhibited more than two-fold greater antioxidant capacity than EO extract. This is apparently due to the presence of all the nonsaponifiable antioxidant compounds of hydrodistilled TQ rich EO as well as additional mixture of phytosterols and other antioxidants, which are also fractionated in ME as NSF during extraction of NS seed powder with methanol. Houghton et al. (1995) have reported that the fixed oil of the NS seed possessed greater antioxidant capacity than TQ. Thus, ME enriched with all the nonsaponifiable DPPH radical scavenging agents of TQ rich EO extract plus good amount of mixture of phytosterols which evidently contributed independently or synergistically with TQ to substantially enhance it’s total antioxidant efficacy.
Lipid peroxidation in food products shares the same chemical mechanism, the free radical-mediated oxidative chain reaction. The products from lipid peroxidation contribute to the rancidity and “off-flavour” of food and consequently cause deterioration in food quality. Some of these products, such as aldehydes, are highly reactive and may also raise concerns of food safety. Antioxidants are added to food to prevent oxidative deterioration during processing and storage. The effectiveness of a selected antioxidant is greatly affected by its physicochemical properties and other components in the food (Frankel et al., 1996b). Because of the consumer preference, novel natural antioxidants, with desired physicochemical properties, are high in demand for food applications to replace synthetic antioxidants, due to their reported carcinogenic activity including BHT and BHA (Onyeneho and Hettiarachchy, 1992). The seeds of black caraway (NS) have been used as a condiment in bread and a spice in cooking for many years, particularly in India, Pakistan, Bangladesh, Italy and southern France (Burits and Bucar, 2000; Nergiz and Otles, 1993). The seeds have also been used to treat fever, headache, cough, asthma, bronchitis, rheumatism, influenza and eczema (Burits and Bucar, 2000; Kumara and Huat, 2001) and have been investigated for their hypoglycemic, antimicrobial, and hypotensive activities (Al-Hader, Aqel, and Hasan, 1993; Hanafy and Hatern, 1991). Therefore, edible NS seeds/oil and its antioxidant extracts, preferably ME, may also have the potential to be developed as natural antioxidant additives to improve the quality, stability and safety of food products, including edible oil blends. In addition, they may serve as excellent dietary source of mixture of natural antioxidants in the protection of biologically important components such as membrane lipids, proteins and DNA from variety of free radical attacks and radical mediated oxidative damage.

Cigarette smoke (CS) has been established as a major risk factor for cardiovascular events such as coronary obstruction, acute myocardial infarction, stroke and sudden death (Barnoya and Glantz, 2005). Furthermore, epidemiological studies have shown that both active and passive or second hand CS increases the risk of atherogenesis (Howard et al., 1998b). In animal studies, inhalation of CS has been reported to promote atherosclerotic plaque development and cardiovascular fibrosis (Kunitomo et al., 2009; Zornoff et al., 2006; Kuo et al., 2005), while environmental
CS created a state of permanent inflammation and an imbalance in the profile of lipid peroxidation products (Yuan et al., 2007). Cigarette smoke is a complex mixture of over 4800 identified constituents including high concentrations of free radicals, and reactive oxygen and nitrogen species (ROS and RNS), reactive aldehydes, and diverse metals (Pryor and Stone, 1993). Aside from smoke-borne organic radicals, cigarette smoking can also induce endogenous production of ROS including superoxide radical, hydrogen peroxide, and hydroxyl radical in various cells (Naziroglu, 2007a; Naziroglu, 2007b), which in turn propagate lipid peroxidation, and cause serious damage to the membrane and changes in intracellular enzymes, resulting in loss of cell function and cell death (Pompella et al., 1991; Kehrer, 1993). Reactive oxygen species are known to be present or formed in CS impose oxidative stress, promote lipid peroxidation, and consequently perturb the antioxidant defense systems in blood and tissues of smokers (Pryor and Stone, 1993). Cell membranes being primarily composed of lipids, especially polyunsaturated fatty acids (PUFAs), are particularly susceptible to attack by these free radicals from CS, leading to increased permeability and altered fluidity of the membrane and thereby causing cellular leakage (Cross et al., 1987). Previously published reports demonstrated a significantly higher levels of lipid peroxidation products, namely, conjugated diene (CD), lipid hydroperoxide (LOOH) and malondialdehyde (MDA) in serum, liver, lung, heart and kidney of CS exposed animals (Ashakumary and Vijayammal, 1996; Zhang et al., 2001; Ramesh et al., 2007a; Solak et al., 2005), while Tanriverdi et al. (2006) have reported an enhanced lipid peroxide formation in CS exposed subjects. On the other hand, erythrocytes are extremely susceptible to oxidative damage induced by ROS because they contain hemoglobin and PUFAs which are readily peroxidized (Chung and Wood, 1971; Yilmaz et al., 1997). This excessive lipid peroxidation in erythrocytes causes injury to cell and intracellular membranes and may lead to cell destruction and subsequently cell death (Naziroglu, 2007a; Naziroglu, 2007b). Furthermore, oxidative stress-induced hypercholesterolemia leads to increased cholesterol accumulation in the erythrocytes and endothelial cells thereby activating and enhancing them to produce additional oxygen free radicals (Prasad and Kalra, 1989; Kay, 1991). Thus, exposure of erythrocytes to these prooxidant conditions can lead
to a number of membrane changes including lipid peroxidation (Sato et al., 1995; Moore et al., 1990), protein crosslinking (Moore et al., 1990) and sulfhydryl group oxidation (Soszynski and Bartosz, 1997), resulting subsequently in membrane damage and hemolysis (Sato et al., 1995; Niki et al., 1988). Membrane enzymes such as ATPases are also targets of free radical attack (Moore et al., 1990; Dwight and Hendry, 1996). Decrease in erythrocyte ATPase activities has been found to coincide with pathological changes of other clinical parameters in coronary heart disease (Zhou et al., 1999). The increased lipid peroxidation upon exposure to CS could be responsible for the decrease in the activities of ATPases, since modification of lipid bilayer environment affects lipid–protein interaction leading to derangement of membrane bound enzymes (Rauchova et al., 1995). In addition, it has been shown that free radicals present in CS cause depletion of protein sulfhydryl groups and increase in protein carbonyl formation (Eiserich et al., 1995). Acetaldehyde, which is present in significant concentrations in CS conjugates with SH group containing components and inhibits their action (Sisson et al., 1991). Since the membrane bound ATPases are thiol-dependent enzymes, it is likely that modification of thiol groups within the active sites of these enzymes by the free radicals generated from the CS could be responsible for their inactivation. Na⁺/K⁺-dependent ATPase pumps Na⁺ out of the cell, thereby lowers the intracellular concentration of Na⁺ by causing an inward proton gradient and pumps K⁺ into the cell against concentration gradient, thus maintaining the electrochemical potential gradient across the cell membrane (Lee, 1991). Tulanko et al. (1988) have shown inhibition of Na⁺/K⁺-dependent ATPase and elevation of Na⁺ in arterial cell wall by chronic exposure to CS, which was attributed to the reorganization of lipid bilayer by increased cholesterol/phospholipid ratio. Reduced activity of Na⁺/K⁺-dependent ATPase and an increase in the level of Na⁺ with a concomitant decrease in the level of K⁺ were seen in the brain of rats exposed to CS (Anbarasi et al., 2005). Previously published reports demonstrated that the activities of Na⁺/K⁺-dependent ATPase, Ca⁺-dependent ATPase and Mg²⁺-dependent ATPase were significantly diminished in lung, liver, kidney, heart (Ramesh et al., 2007a) and brain (Anbarasi et al., 2005) of CS exposed rats.
Consistent with the above reports, in the present study, a significant increase in conjugated diene (CD), lipid hydroperoxide (LOOH) and MDA/TBARS, markers of endogenous lipid peroxidation, were observed in plasma, erythrocyte and liver of sub-chronic CS exposed rats. This increase in lipid peroxides could be attributed to the sustained release of ROS and organic free radicals from CS, which are responsible for the oxidative damage of membrane lipids of the above tissues. In addition, intact erythrocytes from CS exposed control (CS-C) rats exhibited a further increase in susceptibility to \( \text{H}_2\text{O}_2 \)-induced MDA release as compared to basal MDA levels. A similar increase in \( \text{H}_2\text{O}_2 \)-induced MDA release of intact erythrocytes from young smokers (25-45 years; > 5 smoking years) has been reported by Ismail et al. (2002). The increased levels of lipid peroxides in plasma of CS exposed rats may be due to decline in the activity and capacity of free radical scavengers and total antioxidants in the blood circulation. Malondialdehyde crosslinking and lipid peroxidation have been suggested to play a role in the immunological destruction of plasma and erythrocyte antioxidants in smokers. Our results show that increase in plasma lipid peroxidation products is associated with a significant decline in total antioxidant capacity of plasma, as shown by reduced ferric reducing ability (FRAP) and reduced arylesterase antioxidant enzyme activity, indicating an existence of profound oxidative stress in plasma of CS-C rats (Fig. 3.2). These results are consistent with earlier findings (Thirumalai et al., 2010; Cay et al., 2009; Pasupathi et al., 2009), where a significant inverse correlation between the serum/plasma concentrations of MDA and vitamins C, E and \( \beta \)-carotene has been reported in smokers/CS exposed animals. However, in plasma, erythrocyte and liver, protective effects of lipid lowering agents with potent \textit{in vivo} and \textit{in vitro} antioxidant property, such as, TQ and phytosterols rich ME and TQ rich EO extract of NS seeds, pure TQ and pure LMN, were not reported in CS exposed hyperlipidemic rats. Feeding of ME, EO, pure TQ or pure LMN 30 min before exposure of CS to rats effectively reduced the plasma, erythrocyte and liver \textit{ex vivo} CD, LOOH and MDA levels including \( \text{H}_2\text{O}_2 \)-induced MDA release in intact erythrocytes. As expected, overall plasma antioxidant capacity toward FRAP and arylesterase enzyme activity was significantly increased in four treated groups, in the order ME > pure TQ > EO > pure LMN. It is well known that plasma arylesterase or paraoxonase (PON1) activity
is reduced in heavy smokers (Solak et al., 2005), while CS extracts inhibited the PON1 activity in healthy subjects in a time and dose dependent manner (Blatter et al., 1993; Nishio and Watanabe, 1997). In addition, CS has been shown to be associated with a decline in both PON1 activity and concentration in serum of patients with CHD (James et al., 2000; Macknes et al., 2001). Consistent with these reports, in the present study, plasma arylesterase activity was also significantly lower in the CS exposed hyperlipidemic rats. Thus, it would appear that this decrease in arylesterase activity in CS-C rats was primarily due to enhanced prooxidant effect against plasma LDL oxidation and MDA formation. The decrease in HDL-associated arylesterase antioxidant enzyme activity in dyslipidemic rats is consistent with a low but a significant decrease in plasma HDL-C (Fig. 3.10, panel A). Simultaneous supplementation of ME, EO, pure TQ or pure LMN to CS exposed rats caused a significant increase in the plasma levels of both arylesterase activity and HDL-C with a concomitant decline in lipid peroxidation products (Fig. 3.2), and restored their levels higher or close to corresponding control values in N-C. These results suggest that arylesterase/PON being the major HDL component may play a pivotal role in the antioxidative/anti inflammatory/antiatherosclerotic properties of HDL (Aviram et al., 1998).

Consistent with increased oxidative stress in CS exposed rats, our results also demonstrate that both enzymatic and nonenzymatic antioxidant defense systems in erythrocytes and liver fail to neutralize the high amounts of ROS formed with resultant lipid peroxidative damage (Fig. 1.2). Superoxide dismutase (SOD) and catalase (CAT) are antioxidant enzymes that protect the cellular constituents against oxidative damage. Superoxide dismutase is the first enzyme in antioxidant defense that scavenges superoxide radicals to form H$_2$O$_2$ and hence diminishes the toxic effects of the radical. The quinone-semiquinone radicals from the tar phase of CS are capable of reducing molecular oxygen to superoxide radicals whose excessive generation inactivates this enzyme. Hence, a decrease in SOD activity upon smoke exposure could have resulted from its inactivation by tar phase oxidants. Catalase is involved in the detoxification of high concentrations of H$_2$O$_2$. Catalase has been suggested to play an important role in the protection against oxidative damage of tissues (Scibior et al., 2008). The presence and production of the free radicals from
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smoke lower this enzyme, leading to accumulation of H₂O₂ and lipid hydroperoxides, which further enhances the tissue damage. The tripeptide γ-glutamylcysteinylglycine (GSH) is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types at millimolar concentration (Meister, 1989). This cysteine-containing tripeptide exists either in reduced (GSH) or oxidized glutathione (GSSG) form, better referred to as glutathione disulfide, and participates in redox reactions by the reversible oxidation of its active thiol (Briviba et al., 1999). Glutathione usually the most abundant intracellular thiol, plays a key role in protecting cells against electrophiles and free radicals. Glutathione protects against oxidative damage in systems that scavenge radicals, eliminate lipid peroxidation products, preserve thiol-disulfide status of proteins, and repair oxidant damage (Shan et al., 1990). Maintenance of normal cellular GSH levels is crucial for a variety of cellular functions, and oxidative stress has been associated with a decrease in these levels (Okabe et al., 1994). The CS induced depletion of GSH level was directly associated with elevation in lipid peroxidation, which could be attributed to its protection against ROS and free radicals besides its utilization by the antioxidant enzyme, glutathione peroxidase (Gpx). Acetaldehyde, a major aldehyde from the smoke has been shown to deplete the cells of their GSH by conjugating with it, which further makes the cells more vulnerable to peroxidative damage. In addition, the GSH depletion also affects GSH-dependent enzymes such as Gpx, glutathione reductase (Gred) and glutathione-S-transferase (GST) making the cells more susceptible to further oxidative damage. Glutathione peroxidase is widely distributed in almost all tissues. The predominant subcellular distribution is in the cytosol and mitochondrion. This suggests that Gpx is the main scavenger of H₂O₂ in these subcellular compartments. Glutathione peroxidase catalyses the reduction of H₂O₂ and organic hydroperoxides with simultaneous oxidation of GSH to potentially highly cytotoxic GSSG. Thus, both GSH and H₂O₂ act as twin substrates for Gpx. Glutathione reductase is an important enzyme for maintaining the intracellular level of GSH. Glutathione is formed from its oxidized form, GSSG, by the enzyme Gred, which requires NADPH as a cofactor (Meister, 1992; Meister and Anderson, 1983). Glutathione-S-transferase is involved in the detoxification of ROS and tobacco smoke carcinogens, including monohalomethanes, ethylene oxide, dichloromethane,
and the polycyclic aromatic hydrocarbons from cigarette tar, by conjugating them to GSH (Miller et al., 2003). Thus, GST provides protection to tissues by the conjugation of xenobiotics to GSH. The conjugation reaction removes some of the intracellular GSH that may enhance oxidant injury because the level of GSH is depleted and is not available for antioxidant enzymes such as Gpx. Therefore, as the balance between free radical production and antioxidant defenses is lost, the resultant oxidative stress through a series of events deregulates the cellular functions leading to various pathological conditions. An antioxidant compound might contribute partial or total alleviation of such damage. An impaired ROS scavenging function has been linked to the decreased activity of enzymatic and nonenzymatic scavengers of free radicals (Fig. 1.2).

In the present study, a significant decrease in SOD, CAT, Gred and GST (hepatic only) activities along with both free and membrane-linked sulfhydryl groups of GSH, coupled with an increase in Gpx activity, GSSG and GSSG/GSH ratio levels of erythrocyte and liver may be due to their increased utilization to combat the excessive oxidative stress in CS exposed rats. Our results showing an increased erythrocyte and hepatic Gpx activity in CS stressed rats apparently further reduces the GSH content, which is essential for the conjugation of lipid peroxides. In addition, the increased Gpx activity represents a compensatory mechanism to degrade cytotoxic H$_2$O$_2$, which in turn may be an adaptive response to increased oxidative stress due to CS (McCusker and Hoidal, 1990). On the other hand, the decreased activity of GST in liver of CS exposed animals may be due to reduced GSH content and high levels of lipid peroxidation. A similar decrease in enzymatic activities of hepatic SOD, CAT, Gred, and an increase in Gpx has been reported in rats exposed to CS for 90 days (Helen and Vijayammal, 1997), however, contrary to our results, a significant increase in liver GSH content was reported. In an another study in erythrocytes from CS exposed rats a decline in SOD and Gred activities has been reported (Cay et al., 2009). Simultaneous administration of ME or EO fraction from NS seeds, pure TQ or pure LMN to CS exposed rats significantly increased the erythrocyte and liver SOD, CAT and Gred activities as well as GSH (both free and membrane-bound SH groups) concentrations coupled with a decrease in Gpx
activity, GSSG content, GSSG:GSH ratio, and MDA levels and restored them close to corresponding normal control values (Fig. 1.2).

In order to evaluate the response of normal and CS exposed erythrocytes to oxidative stress in vitro, we incubated them with tert-butyl hydroperoxide (t-BHP). As expected, the susceptibility of CS stressed erythrocytes to oxidation was markedly higher compared with normal erythrocytes, as evidenced by a significant decreases of 24% and 50% in free SH and 24% and 43% in membrane-bound SH groups of glutathione with a concomitant increase of 77% and 130% in GSSG, which resulted in a substantial increase of 134% and 364% in GSSG:GSH ratio values (Fig. 3.7). Thus, consistent with in vivo results, when challenged with increased in vitro oxidative stress, CS exposed erythrocytes show markedly decreased antioxidative capacity than normal erythrocytes. The presence of 5 nM pure TQ in the incubation medium significantly protected both normal and CS stressed erythrocytes from t-BHP-induced oxidation of free and membrane (protein)-bound SH groups, GSSG and GSSG:GSH ratio values, and restored them close to corresponding control (minus TQ) values in erythrocytes from N-C and CS-C rats (Fig. 3.7). Consistent with these results, a significantly higher reduction was observed in the activities of total, Mg\textsuperscript{++} and Na\textsuperscript{+} / K\textsuperscript{+}-dependent ATPases in t-BHP exposed erythrocytes from CS-C than N-C rats. Thymoquinone, a principal constituent of EO fraction of NS seeds, with potent antioxidant activity, at 5nM, significantly blocked these decreases in ATPase activities and restored them close to respective control values in N-C or CS-C (Table 5). These results suggest a potent antioxidant activity of pure TQ at an extremely low concentration, in the lipid environment of erythrocyte membranes through its strong peroxyl radical scavenging activity at an early stage of t-BHP-induced free radical attack, thereby rapidly and efficiently protecting the reduced form of glutathione and membrane bound ATPases. In agreement with above findings, incubation of erythrocytes from N-C, CS-C, CS-ME, CS-EO, CS-TQ or CS-LMN groups with both t-BHP and nanogram concentrations of ME, EO, pure TQ or pure LMN exhibited a strong antioxidant protection against t-BHP-induced GSH oxidation and damage of erythrocytes (Fig. 3.5, panel A, B, C and D and 3.6, panel A and B). These results are interpreted to indicate that ME, EO, pure TQ and pure LMN strongly inhibit
lipid peroxidation initiated by free radicals, thus preventing or delaying the damage to cells. In addition, they further reduce the risk of oxidative damage by effectively augmenting both enzymatic and nonenzymatic antioxidants in tissues of CS exposed rats. Elevated SOD, CAT, Gred and GSH along with reduced Gpx, GSSG and GSSG:GSH ratio levels in four treated groups may also be due to elimination of the reactive toxic intermediates, formed as a consequence of excessive oxidative stress, by test fractions/compounds in CS exposed hyperlipidemic rats. The combined in vivo and in vitro results are in complete agreement with our in vitro data (Fig. 3.1), where ME, EO, pure TQ and pure LMN exhibited highly efficient and direct antioxidant effects against DPPH radicals, in the order ME > pure TQ > EO > pure LMN.

Thymoquinone and phytosterols rich methanol and TQ rich EO extracts of NS seeds, pure TQ and pure LMN are shown in the present study to effectively inhibit lipid peroxidation (Table 2), to afford strong protection of both free and membrane (protein)-bound-sulphydryl groups of glutathione (Fig. 3.4) including enzymatic antioxidants (Fig. 3.3) and total, Mg$^{2+}$- and Na$^{+}$/K$^{+}$-dependent ATPases (Table 4), against oxidative damage in erythrocyte membranes of CS exposed rats. Inhibition of ATPases by oxidative attack has been suggested to involve mechanisms including protein crosslinking (Moore et al., 1990), sulphydryl group oxidation (Hebbel et al., 1986; Shao et al., 1995) and lipid peroxidation (Hebbel et al., 1986; Thomas and Reed, 1990; Lam et al., 2007). From our in vivo and in vitro data, it appears that protection of membrane-sulphydryl groups as well as inhibition of both basal and H$_2$O$_2$-induced formation of MDA/TBARS in erythrocyte membranes could explain the protective actions of test fractions/compounds on ATPases against free radical attack. The resultant preservation of membrane structure and function is essential for maintaining membrane fluidity and flexibility as well as ionic balance between the intracellular and extracellular compartments. These processes are crucial for the survival of a cell.

Common feature of CS associated cardiovascular pathology, including atherosclerogenesis and myocardial infarction is associated with impaired lipid and lipoprotein metabolism. Several smoking effects have been described as being atherogenic, such as direct vascular actions (Njolstad et al., 1996), oxidative stress
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(Gouaze et al., 1998), thrombogenic factors (Miller et al., 1998a) and secondary dyslipidemia (Dullaart et al., 1994; Agueda et al., 2004). Cigarette smoke induced dyslipidemia was characterized by a significant increases in plasma TG, TC, VLDL-C and LDL-C including LDL-apoB, while a decline in the cholesterol and apoA-1 levels of antiatherogenic HDL was seen, and this association was dose dependent (Meenakshisundaram et al., 2010; Neki, 2002; Venkatesan et al., 2006; Akbari and Shakoor, 2000; Mammes et al., 2003; Johnkennedy, 2010). It has been suggested that smoking, even of short duration and moderate consumption of cigarettes, is associated with adverse lipoprotein profiles (Raftopoulos and Steinbeck, 1999). Of the apolipoproteins, apoB has been identified in VLDL and LDL and thus appears to be a measure of the total number of atherogenic particles (Elovson et al., 1988). Hence, apoB is a more accurate measure than LDL-C with regard to predicting the CAD. Though both cholesterol and apoB concentrations of LDL particles were significantly increased in mild, moderate and heavy smokers, apoB increase was much stronger. On the other hand, the decline in apoA-1 levels of HDL particles from three categories of smokers had occurred much earlier and was more severe than corresponding levels of HDL-C (Meenakshisundaram et al., 2010). Thus, it is evident that alterations in LDL-apoB and HDL-apoA-1 levels in smokers occur earlier than their corresponding cholesterol contents (Khan and Khan, 1997). Therefore, cardiovascular risk in cigarette smokers was increased due to the enhanced synthesis of LDL associated apoB, which results in a significantly higher level of circulating atherogenic LDL particles (Pastore et al., 2003). Similar observations in lipid profile, such as increase in TG, TC and LDL and decline in HDL were seen among passive smokers (Whig et al., 1992; Neufeld et al., 1997). In addition, cigarette smoking increases the oxidative modification of LDL with circulating products of lipid peroxidation and levels of oxidized LDL found to be significantly increased in both active and passive smokers (Panagiotakos et al., 2004; Heitzer et al., 1996). Epidemiological and laboratory evidence has indicated that smoking by decreasing plasma HDL levels alters the ratios between HDL and LDL, HDL and TG and HDL and TC levels, both in animal models and humans (Yuan et al., 2007; Ambrose and Barua, 2004). Such an alteration in the oxidative stress-linked hyperlipidemia profile of the passive smokers can evidently lead to the
development of CVD, a major part of which is mediated by the circulating serum lipids and specifically the ratio between HDL-C and other harmful circulating lipid transferring factors, such as serum LDL, or TG (Moffatt et al., 2004). Even a short term exposure to passive CS increases the risk of the development of CVD, through the decrease in the circulating levels of HDL and the altered ratio of TC/HDL as demonstrated though experimental studies on humans (Moffatt et al., 2004). These findings suggest that oxidative stress may be a pivotal mechanism for the atherogenic effects of cigarette smoking.

In agreement with above published reports in animals exposed to CS or in both passive and active smokers, our results demonstrate a significant increases in plasma TG, TC, VLDL-C and LDL-C levels with a 11 % (p<0.001) decline in HDL-C in rats exposed to CS for 30 days compared to corresponding values in N-C group. There is abundant evidence that high serum TG independently predict initial CHD events (Austin, 1989; Hokanson and Austin, 1996; Iso et al., 2001; Miller et al., 1998b; Stampfer et al., 1996; Nakanishi et al., 2002), and mild to moderately elevated levels generally considered normal are associated with CHD risk (Castelli, 1992; Miller et al., 1998b; Assmann and Schulte, 1992; Stampfer et al., 1996). In addition, a high serum TG level act as markers for raised concentrations of atherogenic TG-rich lipoproteins, abnormally small dense (sd) particles of LDL, and low serum concentrations of HDL, and the combination of high levels of serum TG, sd-LDL particles, and low HDL-C has been termed as the atherogenic lipoprotein phenotype or simply the lipid triad (Austin et al., 1988; Austin et al., 1990; Grundy, 1998). The increase (117 %) in plasma TG may be attributed to nicotine content of CS, which has been shown to enhance the synthesis of TG in rats administered chronic doses of nicotine (Kavitharaj and Vijayammal, 1999). The increase (89 %) in plasma TC level in CS-C rats is apparently due to increased cholesterol synthesis (Chitra et al., 2000), through the induction (169 %) of hepatic HMG-CoA reductase activity-the rate limiting enzyme in the biosynthetic pathway of cholesterol. The plasma LDL-C was maximally increased by 121 %, followed by VLDL-C (116 %), while increase in the cholesterol concentrations of atherogenic lipoproteins i.e. non-HDL-C (TC-HDL-C) was 119 % in CS exposed rats. As reported earlier (Chen and Loo, 1995) the increase in the cholesterol content of TG rich VLDL might be due to
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CS induced reduction of lipoprotein lipase, which is responsible for the removal of TG from VLDL particles. Thus, the increased level of VLDL-C in CS-C rats is also responsible for high concentrations of atherogenic cholesterol rich circulating LDL. Consistent with previously published reports (Yasue et al., 2006; Porkka and Ehnholm, 1996; Vella et al., 1994; Pugalendi and Ramakrishnan, 1991), high levels of VLDL-C may lead to decreased levels of antiatherogenic HDL-C because of reduced availability of phospholipid remnants for the formation of HDL from VLDL and a concomitant decline in lecithin cholesterol: acyltransferase (LCAT) activity. Consistent with above reports (Yuan et al., 2007; Ambrose and Barua, 2004) in animal models and humans exposed to passive CS, our results revealed a marked decline in the ratios between HDL-C and LDL-C (-60 %), HDL-C and TC (-53 %) and HDL-C and TG (-59 %), which have been established as excellent predictors for both the occurrence and severity of CAD (Drexel et al., 1992). After simultaneous treatment of CS exposed rats with 100 mg ME, 20 mg EO, 10 mg pure TQ or 150 mg pure LMN per rat per day for 30 days, the increases in plasma TG and atherogenic non-HDL-C levels were blocked and significantly reduced, while reduced cholesterol contents of atheroprotective HDL was significantly increased over and above respective normal values in N-C, in the order ME > pure TQ > EO > pure LMN. The data also revealed that ME, EO and pure TQ were most effective in normalizing plasma TC, LDL-C, non-HDL-C, HDL-C/LDL-C, HDL-C/TC and HDL-C/TG ratios to a level, which was 96-98 %, 104-111 %, 97-102 %, 114-127 %, 101-112 %, and 80-88 % of corresponding control values in N-C group, while in pure LMN treated rats, the above parameters were restored by 70-96 % of respective control values. These results indicate that reduction in plasma TC by ME, EO, pure TQ and pure LMN could be attributed to the changes mainly in LDL-C levels. Several epidemiological studies have identified HDL-C as a strong independent cardiovascular risk factor (Assmann and Schulte, 1992; Assmann et al., 1996; Despres et al., 2000), with an estimated 2 % reduction in risk for each 1 % increase in HDL-C (Gordon et al., 1989). A large proportion of patients who suffer a coronary event do not have elevated plasma LDL-C concentrations, but do have low plasma HDL-C and high plasma TG concentrations (Rubins et al., 1995). Therefore, risk reductions, particularly among those patients at high risk, such as diabetics, may
be best achieved by using a combined approach of raising plasma HDL-C and lowering highly atherogenic sd-LDL-C along with LDL-C and TG levels as seen in CS exposed dyslipidemic rats treated with above test fractions/compounds, preferably ME of NS seeds. The present findings indicate that the strong hypocholesterolemic effects of TQ and phytosterols rich ME and TQ rich EO of NS seeds as well as pure TQ and pure LMN, both constituents of EO, are exerted by substantially reducing the fasting plasma TC and LDL-C to normal levels involving a significant suppression of hepatic HMG-CoA reductase activity in the order ME > pure TQ > EO > pure LMN. Methanol extract of NS seeds, rich in both TQ and phytosterols antioxidants, mediated a maximal decline in HMG-CoA reductase activity, from an increased value of 54 units (+169 % vs 20 units in N-C) in CS-C rats to 25 units, which is 81 % of the activity value in N-C. While HMG-CoA reductase activity in CS exposed rats treated with pure TQ, TQ rich EO fraction of NS seeds and pure LMN was down regulated to 77 %, 70 % and 65 %, respectively, of N-C value. Since the average decline and restoration of HMG CoA reductase activity in four treated groups was only 73 % of N-C value, it is difficult to explain highly efficacious lowering of plasma LDL-C, by test fractions/compounds, to an average level of 104 % of normal control value. However, taken together, these data suggest that ME, EO, pure TQ and pure LMN mediated reduction in both plasma TC and LDL-C to normal concentrations might have involved two well known mechanisms: first suppression of liver HMG-CoA reductase mRNA expression, which caused inhibition of cholesterol synthesis and second, increased uptake of LDL-C via up-regulation of LDL receptor gene. These results represent an initial demonstration and provide strong evidence in support of the use of above test fractions/compounds, preferably, ME, as a functional food in the prevention and treatment of passive CS induced dyslipidemia and atherosclerotic complications including CHD.

Several studies have shown a 2- to 3-fold increase in CHD risk in patients with a predominance of sd-LDL particles than those mainly consist of lb-LDL (Austin et al., 1988; Austin et al., 1990; Stampfer et al., 1996). Furthermore, both the prevalence and concentration of more proatherogenic sd-LDL subpopulation was significantly increased in subjects with various types of hyperlipidemia such as
hyperLDLcholesterolemia, hypertriglyceridemia, combined hyperlipidemia and chylomicronemia, hyperlipidemia with CHD, and type 2 diabetic patients with CHD (Hirano et al., 2004). Similarly, Koba et al. (2006) have reported that the progression of CHD was not linked to the LDL particle size, but to the concentration of sd-LDL subfraction. Based on these and other published research more atherogenic sd-LDL has been highlighted as a strong and more precise CHD risk marker (Austin et al., 1995; Hirano et al., 2004; Koba et al., 2006). Consistent with above findings, present study demonstrate that due to enhanced oxidative stress in rats with passive CS induced dyslipidemia, cholesterol and apoB contents of sd-LDL were substantially increased by 337 % and 101 %, compared to N-C values, while these increases in lb-LDL were only 35 % and 19 %. The percent share of LDL in sd-LDL subpopulation was increased from 31 % in N-C to 55 % in CS-C, while in lb-LDL, it was reduced from 69 % to 44 %, indicating a substantial shift in buoyancy from lb-LDL to more atherogenic sd-LDL subpopulation of LDL. Supplementation of ME, EO, pure TQ or pure LMN to CS exposed rats for 30 days fully blocked the shift in buoyancy towards sd-LDL and restored the percent distribution of LDL cholesterol and apoB into sd-LDL and lb-LDL subfractions by an average value of 32 % and 68 %, which are similar to corresponding N-C values of 31 % and 69 %, respectively. It is interesting to mention that in CS-ME treated rats both the cholesterol and apoB concentrations in sd-LDL subspecies were not only fully ameliorated but reduced to a level, which was significantly lower than corresponding normal values in N-C group. These data represent an initial demonstration in CS exposed dyslipidemic rats and provide strong evidence that above test fractions/compounds mediated a specific and substantial decline in more atherogenic sd-LDL subspecies coupled with a significant increase in LDL buoyancy by fully normalizing the plasma concentrations of less atherogenic lb-LDL subpopulation. Together, these results established a highly effective role of test fractions/compounds in the full normalization of atherogenic lipoproteins: the lipid triad, i.e., combination of high plasma TG/TG-rich lipoproteins and sd-LDL subpopulation of LDL as well as low HDL-C levels in the order ME > pure TQ > EO > pure LMN. These findings suggest an important therapeutic use of these extracts/compounds, preferably, ME, in the prevention of cardiovascular risks
associated with hyperlipidemia/dyslipidemia, particularly in people with the sd-LDL phenotype.

Several lines of research have established that exposure to passive CS produces oxidative stress (Howard et al., 1998a; Kosecik et al., 2005; Dietrich et al., 2003; Yildiz et al., 2002; Visioli et al., 2000). In addition to oxidants contained in CS itself (Tribble et al., 1993), free radicals are also released endogenously from activated neutrophils. Importantly, whereas active smokers seem to be adapted to chronic oxidative stress and often exhibit an elevated antioxidant enzyme activity (McCusker and Hoidal, 1990), passive smoking impairs antioxidant mechanisms in non-smokers (Otsuka et al., 2001). In the presence of free radicals and increased oxidative stress due to passive smoke (Harats et al., 1989; Yokode et al., 1995), native LDL is converted to oxidized LDL, which, in turn, elicits a multitude of effects in the vessel wall. Apart from causing endothelial activation and dysfunction (Flavahan, 1992), intramural oxidized LDL is taken up by local macrophages which thereby undergo activation and transformation into foam cells (Glass and Witztum, 2001; Napoli et al., 2001). In humans, elevated levels of thiocyanate, a typical finding in passive smokers (Gasparoni et al., 1998), have been linked to an increased number of macrophages in atherosclerotic plaques (Botti et al., 1996). Moreover, according to experimental data, even short term exposure to passive CS significantly increases lipid accumulation in the wall of perfused rat arteries (Roberts and Rezai, 1996). It is important to mention that polycyclic aromatic hydrocarbons (e.g. benzpyrene), which can be integrated into the plaque after binding to lipoprotein subfractions, also promote the proliferation of vascular cells and plaque progression (Albert et al., 1977; Revis et al., 1984). In vivo studies in animal models of atherosclerosis demonstrated that passive cigarette smoking increases the size and lipid content of lesions in apolipoprotein E-knockout (apoE -/-) mice (Gairola et al., 2001) and in cholesterol-fed rabbits (Zou et al., 2003). Exposure to passive smoke was also shown to change the ultrastructure and mechanical properties of rat pulmonary arteries (Liu and Fung, 1993). Finally, it needs to be mentioned that recent evidence in apoE -/- mice suggests that exposure to passive CS very early in life (e.g. in neonates) may predispose to adult atherogenesis (Yang et al., 2004).
From the above discussion, it is evident that one of the consequences of increased oxidative stress and depleted overall antioxidant defense systems is enhanced lipid peroxidation leading to lipoprotein modification, especially in LDL, which has been postulated to play a central role in initiation of the atherosclerotic process (Tribble, 1999). In the present study it was shown that LDL in the CS exposed dyslipidemic rats had an increased susceptibility to oxidation. This may have resulted from increased oxidative stress, decreased levels of total antioxidants and increased cholesterol content of LDL. In addition, besides increase in the circulating levels of LDL, both the prevalence and concentration of sd-LDL subfraction, which is more atherogenic than lb-LDL subspecies or LDL, was substantially increased due to shift in buoyancy under sustained oxidative stress during CS exposure to animals. Oxidation of LDL is believed to take place mainly in the subintimal space of the arterial wall, because the plasma compartment has an effective antioxidant defense system. Relative to lb-LDL, sd-LDL subpopulation with a much greater tendency to become oxidized might thus be more likely to participate in proatherogenic events in the vessel wall of both passive and active smokers with hyperlipidemia/dyslipidemia, similar to atherogenic lipoprotein phenotype pattern B subjects (De Graaf et al., 1991; Tribble et al., 1992; Dejager et al., 1993; Chait et al., 1993; Tribble et al., 1994; Tribble et al., 1995; Tribble et al., 2001). The *ex vivo* basal levels of conjugated diene (CD) and MDA/TBARS have been suggested to reflect the *in vivo* oxidation of LDL (Planski et al., 1989). To our knowledge, no one to date has reported the excellent protective effects of TQ and phytosterols rich methanol and TQ rich EO extracts of NS seeds, pure TQ and pure LMN, which are shown to exert an effective cholesterol lowering as well as potent *in vivo* and *in vitro* antioxidant property, on the *ex vivo* and *in vitro* Cu**+-induced oxidizability of LDL, it’s more atherogenic sd-LDL, and less atherogenic lb-LDL density subfractions isolated from plasma of CS exposed dyslipidemic rats treated without and with above test fractions/compounds. Our results show that in normal rats, relative to lb-LDL, sd-LDL susceptibility to both *in vivo* and *in vitro* Cu**+-catalyzed oxidation, measured as *ex vivo* basal CD and MDA, or maximal *in vitro* CD and MDA levels were significantly higher by 26 to 31 %. While in comparison to a lag time of 93 min in LDL and 56 min for lb-LDL, the lag time of sd-LDL
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oxidation was markedly reduced to 16 min, indicating a 3.5 to 5.8-fold shortening in the lag time of sd-LDL. Due to exaggerated oxidative stress, CS-induced dyslipidemic rats, in comparison to N-C group, had markedly increased levels of plasma TG/TG-rich lipoproteins and sd-LDL subfraction of LDL and low HDL-C (lipid triad) coupled with depleted concentrations of plasma and tissue enzymatic and nonenzymatic antioxidants including reduced arylesterase antioxidant enzyme activity, known to inhibit oxidation of both LDL and HDL, and ferric reducing ability in plasma, which caused a significantly higher rate of both in vivo and in vitro Cu^{++}-catalyzed oxidation of sd-LDL versus lb-LDL. This is reflected in enhanced (49 % and 52 %) ex vivo basal and maximal CD formation, increased (23 % and 45 %) basal and maximal MDA production and further shortening in lag time (10.5 min in sd-LDL Vs. 40 min in lb-LDL) for oxidation in sd-LDL compared to respective values in lb-LDL. Consistent with these results, maximal CD as well as MDA formation in LDL and lb-LDL was achieved after 2 h of oxidation, while for sd-LDL only 30 min was needed, indicating a substantially increased susceptibility to in vivo and in vitro oxidation of sd-LDL, which may contribute to increased risks associated with atherogenesis. This substantial difference in the oxidative susceptibility between sd-LDL and lb-LDL from normal and CS-induced dyslipidemic rats is consistent with the previous reports indicating an inherently reduced content of free cholesterol and antioxidants, increased amount of more oxidizable polynsaturated fatty acids and preformed hydroperoxide in sd-LDL particles from healthy, combined hyperlipidemic and atherogenic lipoprotein phenotype pattern B subjects (De Graaf et al., 1991; Thomas et al., 1994; Sevanian et al., 1996; Tribble et al., 2001). Our results are also in agreement with an earlier report (Tribble et al., 2001), where intermediate density lipoproteins from subjects with the sd-LDL phenotype had a greater oxidative susceptibility, lower antioxidant concentrations and substantially lower lag time than corresponding particles from subjects with the lb-LDL phenotype. Simultaneous supplementation of methanol or EO extracts of NS seeds, pure TQ or pure LMN, to CS exposed rats, blocked the in vivo and in vitro oxidation of LDL, sd-LDL and lb-LDL, as seen by a significant decrease in ex vivo basal and maximal CD levels and an increase in their lag times, which were restored in ME, EO or pure TQ treated groups to an average value of 89-93 % of
corresponding normal values in N-C rats. Similarly, *ex vivo* basal and Cu^{++}-induced maximal formation of MDA in LDL, sd-LDL and lb-LDL from these treated animals were significantly decreased to near normal levels, which were on the average 82 to 88 % of respective values in N-C group. Pure LMN was least effective as an antioxidant, and was able to restore the above oxidative indices to an average value of 75-76 % of corresponding control values in N-C group. The excellent antioxidant protection of test fractions/compounds afforded to LDL and it’s density subfractions, from *in vivo* and *in vitro* oxidation is consistent with the full amelioration of altered levels of plasma TG/TG-rich lipoproteins, highly atherogenic sd-LDL subpopulation, HDL-C, plasma and tissue enzymatic and nonenzymatic antioxidants including arylesterase antioxidant enzyme activity and ferric reducing efficiency in plasma of CS exposed rats in the order ME > pure TQ > EO > pure LMN.

In summary, *in vitro* studies revealed that methanol and EO extracts of NS seeds had significant and variable antioxidant activities against stable DPPH\. The comparison of scavenging activities of these extracts with pure TQ and LMN, two constituents of EO, against DPPH\(^*\) showed on the average 115 % and 45 % stronger radical-quenching capacity for ME, compared to EO and pure TQ, respectively, in the order ME > pure TQ > EO > pure LMN. These results indicate that ME contained all the radical-scavenging phenolic compounds associated with TQ rich EO fraction of NS seed oil plus good amount of a mixture of phytosterols, known to exhibit strong antioxidant activity, which were also extracted out in methanol as NSF from fixed oil portion of NS seeds. The results from *in vivo* studies demonstrated that oral feeding of methanol or EO extracts of NS seeds, pure TQ or pure LMN 30 min prior to exposure of CS to rats for 30 days effectively reduced the plasma, erythrocyte and liver *ex vivo* lipid peroxidation products, CD, LOOH and MDA including H\(_2\)O\(_2\)-induced MDA release in intact erythrocytes. In addition, overall plasma antioxidant capacity measured as ferric reducing ability and arylesterase antioxidant activity was significantly increased. The results strongly suggest that TQ and phytosterols rich ME and TQ rich EO, pure TQ and LMN protect against oxidative damage to above tissues by preventing excessive lipid peroxidation and by maintaining plasma total antioxidant capacity, erythrocyte and
hepatic enzymatic and nonenzymatic antioxidants at near normal concentrations. The data also demonstrate that protection of membrane-SH groups as well as inhibition of both in vivo, and in vitro H$_2$O$_2$-induced formation of MDA/TBARS in erythrocyte membranes could explain the specific protective actions of test fractions/compounds on total, Mg$^{++}$- and Na$^+/K^+$-dependant ATPases against free radical attack, in the order ME > pure TQ > EO > pure LMN. Consistent with in vivo results, when challenged with increased in vitro t-BHP-induced oxidative stress, erythrocytes from CS exposed rats showed a markedly decreased antioxidant capacity than normal erythrocytes, as evidenced by significantly higher decreases in free, membrane-bound SH groups and total, Mg$^{++}$- and Na$^+/K^+$-dependent ATPase activities in CS-C erythrocytes. Thymoquinone, at 5nM, significantly blocked these decreases, indicating a potent antioxidant activity at an extremely low concentration in the lipid environment of erythrocyte membranes through it’s strong peroxyl radical scavenging activity at an early stage of t-BHP-induced free radical attack, thereby rapidly and efficaciously protecting the reduced glutathione and membrane-bound ATPases. In agreement with above findings, incubation of erythrocytes from N-C, CS-C, CS-ME, CS-EO, CS-TQ or CS-LMN groups with both t-BHP and nanogram concentrations of ME, EO, pure TQ or pure LMN exhibited a strong antioxidant protection against t-BHP-induced GSH oxidation and damage of erythrocytes. The combined in vivo and in vitro results are in complete agreement with our in vitro data (Fig. 3.1) where ME, EO, pure TQ and pure LMN exhibited highly efficient and direct antioxidant effects against DPPH radicals in the order ME > pure TQ > EO > pure LMN. Thus, they play an important role in the preservation of membrane structure and function, which is essential for maintaining membrane fluidity and flexibility as well as ionic balance between intracellular and extracellular compartments, crucial for the survival of a cell.

Besides, exhibiting a highly potent and efficient in vitro and in vivo antioxidant activities, administration of ME, EO, pure TQ or pure LMN to CS exposed rats exerted a strong hypolipidemic effect by reducing plasma TG, TC, VLDL-C, LDL-C as well as proatherogenic sd-LDL and less atherogenic lb-LDL subfractions of LDL to near normal levels, while a significant increase in the concentrations of atheroprotective HDL-C was seen, which was in the order ME >
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pure TQ > EO extract > pure LMN. Our results provide strong evidence that CS-induced hypercholesterolemia elicited a significant increase in hepatic HMG-CoA reductase activity coupled with an increase in plasma TC, particularly in LDL-C and highly atherogenic sd-LDL-C levels. Treatment of CS exposed rats with test fractions/compounds significantly reduced the plasma cholesterol levels by effectively down regulating the HMG-CoA reductase activity. Consistent with above results, substantial reduction in oxidative stress-linked hyperlipidemia in CS exposed rats treated with ME, EO, pure TQ or LMN completely blocked the shift in buoyancy from less atherogenic lb-LDL to more atherogenic and highly oxidizable sd-LDL particles and restored the percent distribution of both cholesterol and apoB of LDL into sd-LDL and lb-LDL subfractions to near normal levels. In addition, relative to lb-LDL, a markedly increased in vivo and in vitro Cu++-induced oxidizability of sd-LDL subpopulation was seen during oxidative stress-mediated induction of hyperlipidemia/dyslipidemia, which was significantly blocked in treated rats, as seen by a decrease in ex vivo basal and in vitro maximal CD formation coupled with an increased lag time as well as reduced production of both basal and maximal MDA.

The combined results presented in the thesis provide strong evidence in support of the use of above test fractions, preferably, TQ and phytosterols rich ME of edible NS seeds, as dietary source of natural antioxidants for disease prevention and/or general health promotion through improved nutrition. The NS seed extracts may also be used as natural antioxidative additives to improve the quality, stability and safety of food products, including edible oil blends. In addition, these test fractions/compounds effectively prevented the above oxidative stress-induced adverse alterations in CS exposed rats and ameliorated/normalized all the CVD risk parameters evaluated in the present study. The data from in vitro and in vivo studies strongly suggest that alleviation of ROS related disorders is due to highly effective amelioration of elevated levels of plasma atherogenic TG/TG-rich lipoproteins, highly oxidizable and proatherogenic sd-LDL subspecies of LDL and low levels of atheroprotective HDL-C, i.e., lipid triad, coupled with a strong inhibition of in vitro and in vivo lipid peroxidation in plasma, erythrocytes and liver along with a markedly efficient free radical scavenging property of test fractions/compounds in
the order ME > pure TQ > EO extract > pure LMN. Therefore, these antiperoxidative, hypolipidemic and atheroprotective agents, preferably, TQ and phytosterols rich ME, may be used as functional food in the protection of ROS/free radical induced oxidative damage, hyperlipidemia and atherosclerotic complications including CHD. However, rigorous clinical trials in active and passive hyperlipidemic smokers without and with CHD are needed to establish their antioxidant, lipid lowering and atheroprotective properties. In addition, subchronic or chronic toxicity data should be thoroughly evaluated before the clinical trial to ensure the safe consumption of above test fractions/compounds within the designated dosages.