II.1. Alcoholic hepatitis

II.1.1. Alcohol

Alcohol is a commonly used substance among people all over the world. With its intoxicating effects and potential for abuse. The main component of all alcoholic drinks is ethanol (ethyl alcohol). Ethanol is one of direct hepatotoxins agents, ethanol ingested in high doses impairs tissues by a variety of mechanisms. Oxidative stress plays surely a crucial role. Most alcohol is broken down (i.e., metabolized) in the liver through a series of chemical reactions, known as oxidation reactions, which involve hydrogen and oxygen atoms. In the predominant biological pathway for alcohol metabolism, called the alcohol dehydrogenase pathway (ADH), a second pathway of alcohol metabolism, the microsomal ethanol-oxidizing system (MEOS), is activated by long-term heavy alcohol consumption.

II.1.2. Definition of Alcoholic Hepatitis

Alcoholic hepatitis is an acute inflammation of the liver, accompanied by the destruction of individual liver cells and scarring. It describes liver inflammation caused by drinking alcohol. It is second progressive stage of alcoholic liver disease. Though alcoholic hepatitis is most likely to occur in people
who drink heavily over many years, but the relationship between drinking and alcoholic hepatitis is complex.\textsuperscript{18}

\section*{II.1.3. Etiology}

Although Alcoholic liver disease has been known since antiquity, the precise mechanism of alcoholic liver disease remains in dispute. Genetic, environmental, nutritional, metabolic, and immunologic factors, as well as cytokines and viral disease have been invoked.

\subsection*{II.1.3.a. Ethanol metabolism}

Most tissues of the body, including the skeletal muscles, contain the necessary enzymes for the oxidative or no oxidative metabolism of ethanol. However, the major site of ethanol metabolism is the liver. Within the liver, three enzyme systems, these enzymes are:-

1. Cytosolic alcohol dehydrogenase (ADH) uses nicotinamide adenine dinucleotide (NAD) as an oxidizing agent.
2. The microsomal ethanol-oxidizing system (MEOS) uses nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen. The central enzyme of MEOS is cytochrome P-450 2E1 (CYP2E1). Ethanol up regulates CYP2E1, and the proportion of alcohol metabolized via this pathway increases with the severity and duration of alcohol use.
3. Peroxisomal catalase uses hydrogen peroxide as an oxidizing agent.\textsuperscript{19}
II.1.3.b. Genetic factors

Although the evidence to prove a genetic predilection to alcoholism is adequate, the role of genetic factors in determining susceptibility to alcoholic liver injury is much less clear. The fact remains that only a small fraction of even heavy alcoholics develop severe liver disease (i.e., cirrhosis). The studies have yielded inconsistent results, as have studies of genetic polymorphisms of collagen, ADH, ALDH, and CYP2E1. The genetic factor that most clearly affects susceptibility is sex. For a given level of ethanol intake, women are more susceptible than men to developing alcoholic liver disease.  

II.1.3.c. Malnutrition

In the past, nutritional deficiencies were assumed to play a major role in the development of liver injury. This view changed after key studies, which demonstrated that alcohol ingestion could lead to steato hepatitis and cirrhosis in the presence of a nutritionally complete diet. However, subsequent studies have suggested that enteral or parenteral nutritional supplementation in patients with alcoholic hepatitis may improve survival.

II.1.4. Diagnosis

II.1.4.1. Clinical symptoms

Although clinical jaundice, hyperbilirubinemia is present in almost every patient with AH and is considered a cardinal feature of this disease. Other symptoms reported in AH patients include right upper quadrant pain, fever,
Tender enlarged liver, other non-specific symptoms may be associated, that include nausea, vomiting, malaise and anorexia. This symptoms may include patients may have associated complications such as hepatic encephalopathy, hepatorenal syndrome, ascites.\textsuperscript{22}

II.1.4.2. Laboratory diagnosis of Alcoholic hepatitis

II.1.4.2a. Laboratory tests

Laboratory tests show increases in serum aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT), Alkaline Phosphatase (ALP), while the increase in alanine aminotransferase (ALT) is less pronounced. Other markers include Hypoalbuminemia, Prolonged Prothrombin Time (PT), increase in Mean Corpuscular Volume (MCV).\textsuperscript{23}

II.1.4.2b. Liver Biopsy

Alcoholic hepatitis usually is diagnosed when a liver biopsy indicates inflammatory changes, liver degeneration, fibrosis, and other changes to liver cells. The 2010 American Association for the Study of Liver Diseases (AASLD) alcoholic liver disease (ALD) practice guideline recommends considering liver biopsy for patients whose diagnosis is reasonably uncertain and for patients likely to undergo medical treatment for severe alcoholic hepatitis.\textsuperscript{24} Alcoholic hepatitis is characterized by hepatocellular injury. Some hepatocytes contain fat droplets (steatosis, green arrow), whereas others may contain intracellular, amorphous, eosinophilic inclusion bodies called Mallory bodies (read arrow), which are often surrounded by neutrophils (yellow arrow) (hematoxylin and eosin)\textsuperscript{25} (Figure 1).
II.1.5. Pathogenesis of alcoholic hepatitis:

II.1.5.1. Mechanism of alcohol-induced hepatic steatosis.

Macrovesicular steatosis occurs in all drinkers within a few weeks of drinking. A combination of increased lipogenesis and impaired fatty acid breakdown results in steatosis which worsens as the alcohol liver disease (ALD) progresses. In hepatocytes the most important pathway is oxidation of ethanol via alcohol dehydrogenase (ADH) to acetaldehyde. In mitochondria, acetaldehyde is converted by aldehyde dehydrogenase (ALDH) to acetate and in turn acetate is converted to Acetyl Coenzyme A (acetyl CoA) which leads the two-carbon molecule into the TCA (tricarboxylic acid cycle). This oxidation generates reducing equivalents, primarily reduced nicotinamide adenine dinucleotide (NAD), which contribute to reactive oxygen species production (ROS). The changes in the NADH–NAD+ potential in the liver inhibit both the oxidation of
triglycerides, fatty acids and the TCA and may thereby increase lipogenesis (Figure 3C3). 26

S-adenosylmethionine (SAMe) is the primary methyl donor and precursor to glutathione. Oxidative stress activation via long term alcohol consumption, through the activity of cytochrome P-450 2E1, that alters the intracellular balance between levels of S-adenosylmethionine (SAMe) and S-adenosylhomocysteine (SAH), resulting in a decrease in hepatic methionine levels, decrease in the activity of methionine adenosyl transferase, the enzyme which converts methionine to SAMe 27 and decrease in the ratio of S-adenosylmethionine to S-adenosylhomocysteine. Decrease in these agents may contribute to alcohol induced liver injury, since S-adenosylhomocysteine exacerbates TNF-α hepatotoxicity, whereas S-adenosylmethionine diminishes it. Reduction in the enzymes that convert (S-adenosylhomocysteine(SAH) to methionine , then convert methionine to S-adenosylmethionine (SAMe) ), lead to increase in homocysteine.

This causes a decrease in glutathione synthesis which results in impaired clearance of oxidative species such as 4-hydroxynonenal (marker of lipid peroxidation) and mitochondrial injury caused by decrease in Glutathione transport from the cytosol into the mitochondria. Increases in concentration of S-adenosylhomocysteine (SAH), stressing the endoplasmic reticulum (ER stress), that lead to release of Sterol regulatory element-binding protein 1c (SREBP-1c) from ER stress. 28

Also ethanol increase in fatty acid synthesis by inhibiting AMP kinase (AMPK or 5’ adenosine monophosphate activated protein kinase). 26 Inhibition of
AMP-activated protein kinase (AMPK) activates hepatic fatty acid synthesis by activating hepatic sterol regulatory element-binding protein (SREBP-1c) transcription. These mechanisms lead to SREBP-1c initiates the transcription of genes involved in triglyceride and fatty-acid synthesis. Ethanol has also been shown promotes lipid metabolism by inhibiting peroxisome-proliferator–activated receptor α (PPAR-α) Decrease in binding of peroxisome-proliferator–activated receptor α (PPAR-α) to DNA reduces the expression of genes involved in fatty acid oxidation. (Figure 3c3)

II.1.5.2. Liver injury due to alcohol metabolites

Hepatocytes convert ethanol to acetaldehyde through three mechanisms: Alcohol dehydrogenase (ADH), cytochrome P-450 isoenzyme-1 (CYP2E1), and catalase. Lipid-peroxidation products can combine with acetaldehyde and with proteins to produce neoantigens, which can stimulate an autoimmune response. Against the background of steatosis and existing liver damage, heavy and continued drinking in some patients causes Alcohol hepatitis. Two main mechanisms are involved: inflammation and oxidative stress.

II.1.5.2.a Mechanism of alcohol-induced hepatic inflammation

Inflammation is a localized defensive response to tissue injury. Liver inflammation is the hallmark of alcoholic hepatitis. Alcohol hepatitis is a condition with features similar to systemic inflammatory response syndrome. Initiating events include expression of gut-derived lipopolysaccharides (LPS),
interaction of LPS with toll-like receptor (TLR4) receptors, activation of inflammatory signaling pathways, Kupffer cells activates and cytokine release. Lipopolysaccharide (LPS), a component of the outer membrane of gram negative bacteria interacts with immune cells and triggers inflammatory reactions with release of cytokines. (Figure 3B1, 2). Alcohol exposure increases gut permeability and facilitates translocation of LPS endotoxin from the small and large intestines to the portal vein and on to the liver (Figure 3A). Increased levels of LPS endotoxins and increase in gut permeability have been shown in patients with alcoholic liver disease.\(^{31}\) when LPS–endotoxin enters portal blood; it becomes bound to LPS-binding protein, a required step for the inflammatory and histopathological responses to alcohol exposure in experimental models. The LPS–LPS-binding protein complex binds with the CD14 receptor on the cell membrane of Kupffer cells in the liver and induces kupffer cells activation. Kupffer cells are pivotal for the development of alcoholic hepatitis.\(^{32}\) the ongoing alcoholinduced LPS absorption may provide a continuing stimulus to Kupffer cells that perpetuates inflammation in alcoholic liver disease. Activation of Kupffer cells by LPS–endotoxin requires three cellular proteins: CD14 (also known as monocyte differentiation antigen), toll-like receptor 4 (TLR4), and a protein called MD2, which associates with TLR4 to bind with LPS–LPS-binding protein.\(^{33}\) (Figure 3B1) Toll-like receptors (TLRs), important components of the innate immune system, function as pattern recognition receptors which recognize and bind proteins and toxins released by pathogens. There are many TLRs, but in alcoholic liver disease TLR4 is most relevant. Interestingly, TLR4 is expressed by
a number of other cells including hepatocytes, hepatic stellate cells, and sinusoidal epithelial cells which may further contribute to alcohol liver disease (ALD). Thus, TLR4 up regulation, in response to endotoxins, prompts Kupffer cells to release large amounts of TNF-α and NF-κB. The downstream pathways of TLR4 signaling include activation of early growth response1 (EGR1), transcription factor (extracellular-signal-regulated kinase 1/2 or ERK1/2), nuclear factor-κB (NF-κB), and the TLR4 adapter known as toll-interleukin-1–receptor domain-containing adapter-inducing interferon-beta (TRIF). EGR1 plays a key role in lipopolysaccharide-stimulated TNF-α production, its absence prevents alcohol-induced liver injury (Figure 3B2). The TRIF pathway (My88-independent) activates NF-κB. NF-κB is a transcription factor that is translocated to the nucleus in response to stress signals and binds to the promoter region of pro-inflammatory, thereby activating multiple cytokine genes. These cytokines including TNF-α, IFN-β, IL-8 and MCP-1 production. Patients with alcoholic hepatitis frequently have high levels of cytokines in their bloodstream, including tumor necrosis factor alpha (TNF-α). TNF-α, produced primarily by Kupffer cells, may cause liver injury directly or indirectly. First, evidence suggests that TNF-α might be directly toxic to liver cells. Second, TNF-α stimulates the liver to produce other cytokines, which attract white blood cells to the liver and stimulate them to release free radicals and toxic enzymes. In experimental animals, TNF-α increase in the liver after 1 month of alcohol administration, timing that coincides with the onset of liver cell necrosis and inflammation. TNF–α production also leads to increased production of chemokines (e.g., IL–8), which attract
inflammatory cells from the bloodstream to the liver, contributing to liver inflammation. Interleukin-8 (IL-8) and monocyte chemotactic protein 1 (MCP-1) have been shown to attract neutrophils and macrophages.\textsuperscript{31} The inflammatory process begins when liver cells release chemicals that attract specialized white blood cells, or phagocytes, to the damaged tissue.

Alcohol is broken down (i.e., metabolized) in the liver cells by two enzymes, alcohol dehydrogenase (ALD) and, particularly after chronic alcohol consumption, cytochrome P450 2E1 (CYP2E1). Both enzymes convert alcohol to acetaldehyde, a toxic substance. Some of the acetaldehyde interacts with proteins in the cells such as malondialdehyde, which results from lipid peroxidation, interact, through a covalent binding, with the reactive lysine residues of proteins, formation malondialdehyde-acetaldehyde proteins adducts (MAA) which called adducts, located on the membranes of hepatocytes (figure 3C2). MAA proteins adducts capable to produce neoantigens, which can activate certain immune cells to produce various cytokines, including interleukins such as (IL–1, IL–2), interferon gamma (IFN–γ), and tumor necrosis factor alpha (TNF–α), that in turn attacks healthy liver cells, resulting in tissue damage.\textsuperscript{36,37}

There are other sources of oxidative stress caused by alcohol to induce inflammation, that include granulocytes from the inflammatory process catalyzed by the enzymes NADPH oxidase and myeloperoxidase which cause generates highly reactive molecules known as reactive oxygen species (ROS), which accumulate primarily in cell structures called mitochondria.\textsuperscript{38} ROS normally are eliminated from the cells by compounds known as antioxidants, particularly a
small molecule called glutathione (GSH). Alcohol, however, depletes the cell’s GSH stores, thereby further exacerbating ROS accumulation in the mitochondria. This process leads to the release of cytochrome c from the mitochondria, which then activates enzymes called caspases and promotes production of IL-8 in the cell. ROS can also activate ERK1/2, p38 MAPK kinases, and NF-kB which stimulates TNF-α, complementing the LPS-induced pathways of TNF-α production and creating a vicious cycle of inflammation and oxidative stress.

II.1.5.3. Mechanism of alcohol-induced mallory bodies

In hepatocyte, ethanol is converted to acetaldehyde by the cytosolic enzyme alcohol dehydrogenase (ADH) and the microsomal enzyme cytochrome P-450 2E1 (CYP2E1). CYP2E1 may increase 5-20 fold in patients with alcoholic hepatitis, leading to increased electron leakage and release of ROS by CYP2E1 and mitochondria cause lipid peroxidation and produce protein carbonyls, that lead to inhibition of proteasomes. Inhibition of the proteosome reduces the catabolism of damaged proteins and may contribute to the accumulation of abnormal form of ubiquitin called Ub+1. Ubiquitin (Ub+1) is formed when the gene encoding ubiquitin is “misread” by the cell’s machinery, resulting in synthesis of ubiquitin molecules that are longer than normal. When these abnormal ubiquitin-protein conjugates called cytokeratin filaments, they cannot be removed as easily as normal ubiquitin, thereby slowing down the degradation of the attached protein, that lead to accumulation of cytokeratin filaments, and formation of Mallory bodies on liver histology. In addition, Ub+1 itself inhibits proteasome function and could thereby cause liver cell death. (figure 3C1)
**Figur 2&3 (A,B,C1,C2,C3)** Overview of the pathway of alcoholic injury resulting in the direct production of oxidative stress and the LPS-TLR pathway that culminates in production of cytokines and other inflammatory processes that result in hepatitis of the liver. (Adapted from: Michael R. Lucey M R, Mathurin P and Morgan T R.)
II.2. *Nigella Sativa* Seeds

II.2.1. Description

An annual herbaceous plant (*Nigella sativa*), the plant has finely divided foliage and pale bluish purple or white flowers. The flowers grow terminally on its branches while the leaves grow opposite each other in pairs, on either side of the stem. Its lower leaves are small and petioled, and the upper leaves are long (6-10cm). The stalk of the plant reaches a height of twelve to eighteen inches as its fruit, the black seed, matures. *Nigella sativa* is bisexual and forms a fruit capsule which consists of many white trigonal seeds. Once the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in color (black seeds). *Nigella sativa* and its black seed are known by other names, varying between places. 41 (figure 4)

Figure 4: Black Seed Flower, black Seed (*Nigella seeds: the miraculous Black Seed.  (Adapted from:  http://www.healthinfo21.com/2010/11/nigella-seeds-miraculous_black-seed.html). 42
Nigella sativa Scientific classification: Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Ranunculales, Family: Ranunculaceae, Genus: Nigella, Species, Nigella sativa. Scientific name is a derivative of Latin niger (black). Original black cumin seed is Carum bulbocastanum. The botanical name is Nigella sativa, while in English, Nigella sativa seed is variously called fennel flower, nutmeg flower, Roman coriander, black seed or black caraway. It has Other names such as habbat al-barakah (Arabic), kaljeera (Assamese), kalo jira (Bengali), karum cheerakam (Tamil), kalonji (Hindi/Urdu) or mangrail (Hindi), ketzakh (Hebrew), chernushka (Russian), çörek otu (Turkish), siyah daneh (Persian), karim jeerakam (Malayalam or in Sinhala).

43
II.2.2. The Chemical Composition of the Seeds

The general chemical composition of the seeds is depicted in Table 2.

**Table 2: The general chemical composition of *Nigella sativa* seeds**

Constituent % Range (w/w). (Adapted from: Ismail M Y M. )

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% Range (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>31-35.5</td>
</tr>
<tr>
<td>Protein</td>
<td>16-19.9</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>33-34</td>
</tr>
<tr>
<td>Fibre</td>
<td>4.5-6.5</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.013</td>
</tr>
<tr>
<td>Moisture</td>
<td>5-7</td>
</tr>
</tbody>
</table>

II.2.3a. Chemical Composition of *N. sativa* Oil

The chemical analysis of *Nigella sativa* total oil revealed the presence of both a fixed oil and a volatile oil. The major component was the fixed oil whereas the volatile oil ranged from 0.4- 0.7% of the seeds’ weight. The fixed oil chemical composition is outlined in Table 3.
Table 3: The chemical composition of *Nigella sativa* fixed oil. (adapted from :- Ismail M Y M.)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic Acid</td>
<td>44.7-56</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>20.7-24.6</td>
</tr>
<tr>
<td>Linolenic Acid</td>
<td>0.6-1.8</td>
</tr>
<tr>
<td>Arachidic Acid</td>
<td>2-3</td>
</tr>
<tr>
<td>Palmitoleic Acid</td>
<td>3</td>
</tr>
<tr>
<td>Eicosadienoic Acid</td>
<td>2-2.5</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>12-14.3</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>2.7-3</td>
</tr>
<tr>
<td>Myristic Acid</td>
<td>0.16</td>
</tr>
<tr>
<td>Sterols</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Generally, there were no significant variations in the chemical composition of the fixed oils of seeds grown in Egypt, Sudan, Ethiopia, India, Turkey and Syria. However, Al-Jassir noted that the seeds grown in Qassim, Saudi Arabia, contained, in addition to the fatty acids depicted in Table 2, two more acids which
were lignoceric acid about (1%) and myristoleic acid (0.18%) without the presence of eicosadienoic acid (C20:2). Lignoceric acid is not found in many other edible vegetable oils. Specific chemical analyses of the volatile oil started by Mahfouz and El-Dakhakhny and Canonica et al. These studies were complemented by most recent ones which revealed various pharmacologically active constituents that included Thymoquinone that may attain up to 27.8% of the volatile oil (w/w), Carvacrol phenol which is also known as isothymol (5.8-11.6% (w/w)), p-cymene in the range of (15.5-31.7% (w/w)), α-pinene in the range of (9.3% (w/w)), α-terpineol in the range (2-6.6% (w/w), longifolene (1-8% (w/w)), t-anethole (p-Propenyl anisole)benzene in range (0.25-2.3% (w/w)) and the reduction product of thymoquinonethymohydroquinone together with some esters about 16%.

II.2.3b. Non-Oily Components of Nigella sativa Seeds

1. Minerals

Analysis of Nigella sativa seeds, ash revealed the presence of 0.5-1% calcium, 0.6% phosphorus, 0.6% potassium and 0.1% sodium of the total seeds weight.

2. Saponins

The major saponin in the defatted seeds of Nigella sativa is the glycoside α-hederin or Helixin or melanthin which on acid hydrolysis releases its sugar rhamnose / arabinose and gives the aglycone hederagenin (or melanthigenin) or caulosapogenin.
3. Alkaloids

Three types of alkaloids were isolated from the defatted seeds of *Nigella sativa*. These were identified as the indazole nigelicine, the isoquinoline nigellimine and its N-oxide and the indazole alkaloid nigellidine.\(^{44}\)

**II.2.4. Therapeutic potential of *Nigella sativa* seeds**

**II.2.4.1. Anti inflammation of *Nigella sativa* seeds**

Inflammation has been known to produce proinflammatory cytokines and diverse reactive oxygen species (ROS) and reactive nitrogen species (RNS) creating pre-disposition to various patho-physiological disorders. The amelioration of the Experimental Allergic Encephalitis by TQ was examined, who showed potent effects, which were thought to occur via induction of glutathione. The effect of Th2 cytokine response were investigated in vitro in lipopolysaccharide (LPS)-activated rat mast cells. Thymoquinone (TQ) significantly inhibited LPS-induced IL-5 and IL-13 mRNA expression and protein production. TQ was found to decrease NF-κβ activation in a dose-dependent manner with maximum inhibitory effect at a concentration of 500nM.

NF-κβ is a molecular target of TQ. Further studies by Mohamed and colleagues in 2005 showed the effects of TQ on the inhibition of activation of NF-κB in an experimental autoimmune encephalomyelitis in the rat model of multiple sclerosis. TQ has been shown inhibit of activation of NF-κβ in an experimental autoimmune encephalomyelitis, TQ was able to increase the red blood cell glutathione, and inhibit the activation of NF-κβ in the brain and spinal cord.
These results clearly provide some early indication that many of the biological activity of TQ could in part be due to inactivation of NF-κB and its downstream genes. Collectively, these results suggest that NF-κB is a molecular target of Thymoquinone.45,46

This study will use Herb of the Mediterranean region originally from Syria having pungent seeds. Three doses of *Nigella sativa* seeds extract are (0.5 g/kg of b.w, 1 g/kg of b.w, 1.5 g/kg of b.w daily) by orally using sonde tube for 8 weeks.

II.2.4.2. Antioxidant Activity of *Nigella sativa* seeds

Both *Nigella sativa* oil and Thymoquinone can partly protect gastric mucosa from acute alcohol-induced mucosal injury which is partly ascribed to their radical scavenging activity. Thymoquinone(TQ) was the main active constituent of volatile oil of the black seed. It has been shown to exhibit antioxidant property through different mechanisms in several recent reports. For example, it inhibits the production of 5-hydroxyeicosa- tetraenoic as well as 5-lipoxygenase products, also it was shown to work as a scavenger of various reactive oxygen species including (ROS), superoxide radical anion and hydroxyl radicals. It has been shown that TQ could inhibit iron-dependent microsomal lipid peroxidation efficiently (MEOS). The compound was observed to decrease cellular oxidative stress by inducing glutathione in experimental allergic encephalomyelitis. Present findings suggest that Thymoquinone has a potent chemopreventive potential of inhibiting the process of carcinogenesis by
modulating lipid peroxidation and cellular antioxidant milieu. TQ have found to react with GSH, NADH and NADPH chemically. Such reactions occurring under the physiological conditions clearly indicate the formation of two products, viz. glutathione dihydrothymoquinone after rapid reaction with GSH and dihydrothymoquinone (DHTQ) after slow reaction with NADH and NADPH, respectively. These compounds exhibit antioxidant capacity. These results indicate a possible intracellular non-enzymatic activation of TQ dependent upon GSH, NADH and NADPH representing perhaps the ‘cellular switch’ for modulating cellular antioxidant defenses. The protective effects of Thymoquinone have been assessed. Results suggest that treatment with Thymoquinone increased GSH levels compared to normal levels and inhibited the in vitro production of superoxide radicals in enzymatic and non-enzymatic systems. Active antioxidant components of black seeds of Nigella sativa plants have been shown capable of rendering protection against the development of methionine-induced hyperhomocysteinemia (Hhcy) and its associated state of oxidative stress. Pretreatment of rats with an oral dose of 100 mg/kg of TQ for 30 min and for one week provided complete protection against induced HHcy after methionine load (100 mg/kg). Treatment with the different doses of Thymoquinone(TQ) produced significant reductions in hepatic SOD, CAT and GSH-Px activities although neither produced any change in GST activity nor influenced reduced glutathione content in any of the tissues studied. These results revealed that Thymoquinone(TQ) and dihydrothymoquinone (DHTQ) acted not only as superoxide anion scavengers but as general free radical scavengers.
The effects of TQ on carbon tetrachloride (CCl4)-induced hepatotoxicity have been investigated in male Swiss albino mice. Oral administration of TQ in a single dose (100 mg/Kg) resulted in a significant protection against the hepatotoxic effects of CCl4. The result suggests that the protective action. The effects of TQ against CCl4-induced hepatotoxicity may be mediated through the combined antioxidant properties of TQ and its metabolite DHTQ.\textsuperscript{45,46}

\textbf{II.2.5. Side Effects of \textit{Nigella sativa} seeds}

Black seed is a safe and effective herb that can be used by almost anyone. No irritations or side effects are caused when the right dose is correctly applied. Its benefits are obtained through consistent use, the effects are medium to long term. Black seed should not be taken by pregnant women if their wombs are sensitive (Many Muslim women take it while pregnant and no harm has been found).\textsuperscript{47}

\textbf{II.3. Animal models of ethanol-induced liver damage}

Animals have been administered ethanol by various methods in attempts to try to develop liver lesions resembling those seen in human ALD. The animals continued to gain weight with ethanol concentrations up to 50\%, showed increased ethanol metabolism, and sustained elevated blood ethanol levels, but liver damage occurred at most in only a minority of the animals. To overcome the failure of most animals otherwise to consume higher amounts of ethanol voluntarily, it has been administered in a nutritionally adequate liquid diet that provided a maximum of 35-40 \% of total calories from alcohol. This situation resembles that of many alcoholics, who often receive more than 50 \% of their total
energy as ethanol. In rats this has been achieved by administration of ethanol as a component of the liquid diet, either orally or by forced intragastric infusion.

II.3.1. Oral liquid diets

The method forces rats to consume high amounts of ethanol by its inclusion in a balanced liquid diet that contains sufficient water and all necessary nutrients. It was developed almost four decades ago and proved to be very useful in studies of the pathogenesis of early ethanol-induced changes.

II.4. Hepatic tissue changes

II.4.1. Effect of Nigella sativa seeds against alcohol-induced hepatic steatosis

In hepatocytes the most important pathway is oxidation of ethanol via alcohol dehydrogenase (ADH) to acetaldehyde. In mitochondria, acetaldehyde is converted to acetate and in turn acetate is converted to acetyl CoA which leads the two-carbon molecule into the TCA (tricarboxylic acid cycle). This oxidation generates reducing equivalents, which leads ultimately to reduced nicotinamide adenine dinucleotide (NAD), which contribute to reactive oxygen species production (ROS). The changes in the NADH–NAD+ potential in the liver inhibit both the oxidation of triglycerides, fatty acids, and may thereby increase lipogenesis.

Thymoquinone (TQ) has found to react with GSH, NADH and NADPH chemically. This reactions lead to form of two products, viz. glutathione
dihydrothymoquinone after rapid reaction with GSH and dihydrothymoquinone (DHTQ) after slow reaction with NADH and NADPH, respectively. These results indicate a possible intracellular non-enzymatic activation of TQ dependent upon GSH, NADH and NADPH representing perhaps the ‘cellular switch’ for modulating cellular antioxidant defenses. In additionally, Thymoquinone was observed to work as general free radical scavengers, These indicators suggests that TQ may able to reduce NADH ,thereby reducing the NADH–NAD+ changes, Which leads ultimately to inhibiting the process of lipogenesis.

Oxidative stress activation via long term alcohol consumption, through the activity of cytochrome P-450 2E1,that alters the intracellular balance between levels of S-adenosylmethionine (SAMe) and S-adenosylhomocysteine (SAH), resulting in a decrease in hepatic methionine levels, decrease in the activity of methionine adenosyl transferase, the enzyme which converts methionine to SAMe ,that lead to increase in S-adenosylhomocysteine (SAH), homocysteine. Increases in concentration of S-adenosylhomocysteine(SAH), stressing the endoplasmic reticulum (ER stress),that lead to release of Sterol regulatory element-binding protein 1c (SREBP-1c) from ER stress. hepatic sterol regulatory element-binding protein (SREBP)-1c initiates the transcription of genes involved in triglyceride and fatty-acid synthesis.

TQ could inhibit iron-dependent microsomal lipid peroxidation efficiently. However the compound was observed to decrease cellular oxidative stress by inducing glutathione. According previous study wich indicated that active antioxidant components of black seeds of Nigella sativa plants capable of
rendering protection against the development of methionine-induced hyperhomocysteinemia (Hhcy) and its associated state of oxidative stress. These results suggests that TQ capable to provide balancing between SAMe and SAH , which leads ultimately to provided protection against the transcription of genes (SREBP-1c) that involved in triglyceride and fatty-acid synthesis.

II. 4. 2. Effect of Nigella sativa seeds against alcohol-induced hepatic inflammation.

Alcohol exposure increases gut permeability and facilitates translocation of LPS endotoxin from the intestines to the portal vein and on to the liver, it becomes bound to LPS-binding protein. The LPS–LPS-binding protein complex binds with the CD14 receptor on the cell membrane of Kupffer cells in the liver and induce kupffer cells activation via three cellular proteins: CD14, TLR4, MD2, which associates with TLR4 to bind with LPS–LPS-binding protein. Interestingly, TLR4 is expressed by a number of other cells including hepatocytes, hepatic stellate cells, and sinusoidal epithelial cells. Thus, TLR4 up regulation, in response to endotoxins, prompts Kupffer cells to release large amounts of TNF-α and NF-κβ. NF-κβ is a transcription factor that is translocated to the nucleus in response to the promoter region of pro-inflammatory, thereby activating multiple cytokine genes, which may further contribute to hepatic inflammations.

Thymoquinone (TQ) was found to increase the red blood cell glutathione, and inhibit the activation of NF-κβ. Where indicates that
Thymoquinone has a role in reducing inflammation through its ability to reduction in amount of cytokines via inhibit activity of NF-κβ.

Alcohol dehydrogenase (ALD) and, particularly after chronic alcohol consumption, cytochrome P450 2E1 (CYP2E1). Both enzymes convert alcohol to acetaldehyde, a toxic substance. Some of the acetaldehyde interacts with proteins in the cells, formation malondialdehyde-acetaldehyde proteins adducts (MAA) which called adducts, located on the membranes of hepatocytes. MAA proteins adducts capable to activate certain immune cells to produce various cytokines, that in turn attacks healthy liver cells, resulting in tissue damage.

TQ have been shown inhibit iron-dependent microsomal lipid peroxidation efficiently. This effect of TQ lead to decrease in malondialdehyde, lipid peroxidation products, thereby lead to inhibition neo antigens production, cytokines activation caused by MAA, which in turn lead to decrease in hepatic inflammation caused by alcohol.

There are other sources of oxidative stress caused by alcohol to induce inflammation, that include granulocytes from the inflammatory process catalyzed by the enzymes NADPH oxidase and myeloperoxidase which cause production of ROS, and a decrease in antioxidants. This process leads to the release of cytochrome c from the mitochondria which then activates enzymes called caspases and promotes production of IL-8 in the cell.

According results that indicate a possible intracellular non-enzymatic activation of Thymoquinone (TQ) dependent upon GSH, NADH and NADPH
representing perhaps the ‘cellular switch’ for modulating cellular antioxidant defenses. also thymoquinone (TQ) was shown to work as a scavenger of various reactive oxygen species including (ROS) superoxide radical anion and hydroxyl radicals . Addition other studies indicates that Thymoquinone have a role in reducing oxidative stress by increasing glutathione . These effects lead to inhibition of generates reactive oxygen species (ROS), that lead to reducing cytochrome c production from the mitochondria which finally lead to reducing inflammation and oxidative stress.

II.4.3. Effect of Nigella sativa seeds against alcohol-induced hepatic mallory bodies.

CYP2E1 may increase in patients with Alcoholic hepatitis, leading to increased in release of ROS by CYP2E1 and mitochondria cause lipid peroxidation and produce protein carbonyls, that lead to inhibition of proteasomes. Inhibition of the proteosome leads ultimately to formation of Mallory bodies on liver histology.

Active component of Nigella sativa seeds of Nigella sativa plants (Thymoquinone) have inhibit iron-dependent microsomal lipid peroxidation efficiently, as will thymoquinone (TQ) was shown to work as a scavenger of various reactive oxygen species including (ROS) superoxide radical anion and hydroxyl radicals, additionally thymoquinone (TQ) was shown increase glutathione (GSH) levels. These effects of black seeds are capable of rendering protection against formation of Mallory bodies on liver histology.
Figure 5. Theoretical framework of the study
II.5. Conceptual framework

![Conceptual framework diagram]

**Figure 6.** Conceptual framework of the study

II.6. Hypothesis

1. There's a difference in terms of expression of TNFα and hepatic tissue damage after administrate with *Nigella sativa* extract among the three groups of treatment (0.5 g/kg of Bw, 1 g/kg of Bw, 1.5 g/kg of Bw and control).