Review of Literature
CHAPTER 2
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CHAPTER 2

REVIEW OF LITERATURE

Hepatic injury

Causes of hepatic injury and hepatotoxicity

Liver, the largest organ in the body is essential in keeping the body functioning properly. It removes or neutralizes poisons from the blood, produces immune agents to control infection and removes microbes from the blood. It makes proteins that regulate blood clotting and produces bile to help absorb fats and fat-soluble vitamins. Because of these activities it is exposed to a wide variety of insults and is therefore one of the most frequently injured organs of the body. Yet one cannot live without a functioning liver (Kirsch et al., 1995).

Hepatic disorders

Injury from metabolic disturbances

In experimental animals, specific dietary deficiencies can produce fatty liver and liver cell necrosis. Similarly in man, protein malnutrition can produce marked fatty changes and there is evidence that malnutrition may considerably exacerbate other forms of injury. Specific enzyme deficiencies may cause various hepatic storage disease, or failure of bile excretion (Glaister, 1986).

Ischaemic injury affects the perivenular zone of the acinus as do many drugs and toxins, for example alcohol and paracetamol. Less commonly, some agents for example, phosphorous produce periportal injury, while yellow fever characteristically affects zone 2 of the acinus, producing a 'mid-zonal' pattern of necrosis (Quiroga et al., 1992).

Hepatic disfunctions

Hepatic metabolic disorders, and involvement of liver in the extrahepatic disorders, lead to hepatic disfunctions.
Hepato metabolic disorders

The problems with metabolic processes in the liver can be either congenital or acquired. Some of the disorders such as Wilson's disease and hemochromatosis are congenital (Sherlock and Summerfield, 1991). A-1-antitrypsin deficiency is an inherent disease acquired due to smoking where 10% of adult patients will develop liver disorders (Quiroga et. al., 1992).

Extrahepatic Disorders

In the extrahepatic disorders liver may be affected by numerous conditions particularly autoimmune disorders in which the immune system attacks the body's own normal tissues. Eg. rheumatic diseases and inflammatory bowel disease. Systemic infections such as tuberculosis can spread to liver (Kirsch, 1995).

Hepatotoxins

Alcoholism

Alcohol consumption is considered to have a major share for the chemically induced liver injury. Alcohol abuse is a leading cause of mortality and morbidity throughout the world and in USA as many as 10% of men and 35% of women suffer from persistent problems of alcohol abuse. The incidence of liver cirrhosis among alcoholics is about 10-15% (Pequignot and Cyrulnik, 1970) and in the United States, alcohol abuse is the ninth leading cause of death (Grant et. al., 1986). In New York City, alcoholic cirrhosis is the third leading cause of death between the age of 25-65 years are the most affected ones (Lieber, 1988).

Therapeutic Drugs

One of the major side effects of drugs is liver injury. It has been recorded that approximately 3% of all hospital admissions are due to adverse drug reactions and 20-30% of the cases of severe hepatic failure are drug-induced (Ward et. al., 1997). Many non-steroidal
antiinflammatory drugs like aspirin, indomethacin, ibufenac and fluproguazone can induce hepatic necrosis (Lewis, 1984). Antibiotics like tetracycline are also known to cause injury mainly necrosis and steatosis (Timbrell, 1983). Paracetamol (acetaminophen) is an analgesic, which at high doses induce hepatic injury (Black, 1980).

**Chemical Toxicants**

Chemical toxicants in the occupational or non-occupational environment are also a common cause of hepatic injury. Exposure to environmental chemicals that leads to chronic liver disease or primary hepatic malignancies has also been documented. Solvents and degreasing agents, pesticides, polyhalogenated biphenyls, dioxins, dibenzofuranes and vinyl chloride are some of the major chemical hepatotoxicants that have evolved as a result of rapid industrialization. Industrial exposure to vinyl chloride monomer and solvents like 1, 1, 1-trichloro ethane are known to cause liver damage and can lead to abnormal levels of transaminases (Tamburro et. al, 1984 and Hodgson et. al, 1989). Short exposure to industrial chemical like dimethyl formamide can result in local hepatocellular necrosis and steatosis (Redlich et. al, 1990). Industrial and occupational liver injury is under diagnosed and the prognosis of the chronically exposed is uncertain.

**Hepatotoxicants induce tissue injury**

**Mediated by phagocytes and inflammatory mediators**

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<thead>
<tr>
<th>Neutrophils</th>
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Microbial Infections

Viral hepatitis

Certain virus infections damage the liver, severely causing acute hepatitis with extensive necrosis of liver cells. These include hepatitis A, hepatitis B, the non-A; non-B group and many others. Progression to chronic hepatitis is a complication of type B and some of the non-A; non-B group.

Fungal Hepatic Injury

In man, acute liver failure is observed following ingestion of mushrooms (fungi) like *Amanita phalloides* and *A. verna*. The toxins present in these mushrooms, namely phalloidin and phalloin are extremely lethal to liver cells (Rensberg, 1977).

Other Infections

In addition to viruses and fungi, bacteria and protozoans can infect the liver. The liver is almost inevitably involved to some extent in all blood-borne infections (Kirsch *et al.*, 1995).

Hepatitis

Viral hepatitis is believed to have been recognized as early as Biblical times. It is a disease noted over the centuries to have occurred mostly in epidemics, in the context of poor hygiene, especially during wartime. That a virus is responsible for the disease was suggested as recently as the turn of this century, and that more than two viruses could cause the illness was suggested only fifty years ago. During the course of the past twenty years, five distinctly separate hepatitis viruses have been identified, all of which have been characterized and now cloned.

Types of hepatitis - Hepatitis A

Hepatitis A virus is a picornavirus and occurs in the blood and faeces 3-4 weeks after exposure to the virus. Infection is very common in
children under 15, with a higher frequency in the lower socio-economic classes.

**Hepatitis B**

Hepatitis B virus is a DNA virus and it is a member of the hepacina group. It is most frequently transmitted by blood and blood products. The virus may also be present in body fluids, saliva, semen and vaginal secretions and may also be transmitted by intimate physical contact including from mother to child and sexually. It is a particular hazard among active male homosexuals and intravenous drugs abusers.

**Hepatitis D**

Previously known as the delta agent this is a defective RNA virus, which usually requires HBV for its replication. It can be acquired simultaneously with HBV (co-infection) or it may secondarily infect chronic HBV carriers (Super-infection). In both cases the clinical illness is more severe and the liver injury more extensive than infection with HBV alone.

**Non-A; Non-B Hepatitis**

The term non-A; non-B hepatitis was used to describe clinical cases of viral hepatitis in which the etiological agent could not be defined. Most of these occurred after blood transfusion and a high proportion led to chronic hepatitis.

**Hepatitis C**

Hepatitis C virus is an RNA virus. It is transmitted by blood and blood products and is now the most important cause of post-transfusion hepatitis. It is prevalent among intravenous drug addicts and c-infection with HBV can occur. The only treatment of proven value is α-interferon.

**Hepatitis E**

The hepatitis E virus is an RNA virus and is transmitted by the faecal-oral route. It mainly affects young adults in whom it causes a mild
illness with jaundice; however, there is high fatality rate in pregnant women.

**Hepatitis -B – the silent killer**

**Epidemiology**

**Incidence / Prevalence**

Hepatitis B virus is a major cause of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide. Prevalence rates vary widely among geographically diverse areas. Approximately 45% of the world's population live in regions of high HBV endemicity. Overall, an estimated 300 million persons are carriers of the virus. In the United States, a region of low endemicity, a survey by the Centers for Disease Control (CDC) identified an overall prevalence of chronic HBV infection of 0.3% among whites and 1.1% among blacks. Because of substantial under reporting, actual incidence rates of new infections are difficult to calculate (Margolis and Hadler, 1991). The incidence of new infections however has not been static. Since the mid-1980s, the number of annual new cases of acute hepatitis B infections reported to the CDC has decreased by approximately 50%.

The risk of acquiring chronic HBV infections varies inversely with the age at which the infection acquired. Chronic infection occurs approximately 90% of infants infected at birth, 25-50% of children infected from 1-5 years of age, but in fewer than 1% of immunocompetent persons infected as adolescents or adults (Alter et. al., 1990). Similarly, because most cases of hepatitis occur in areas of high endemicity and most such cases occur in infants and young children, from a global perspective, persons infected early in life account for the vast majority of cases culminating in end-stage cirrhosis and hepatocellular carcinoma.

**Hepatitis B in India**

Hepatitis B virus (HBV) continues to be the single most important cause of viral hepatitis in the developing and underdeveloped world and
along with hepatitis c virus (HV), is a formidable cause of chronic liver diseases and primary carcinoma of liver, the world over. Hepatitis B is a major cause of morbidity and mortality worldwide. Till date it remains a clinical challenge and a problem of great importance. To date there are nearly 370 million HBV carriers in the world with the highest incidence of 10-20% in tropical countries. India is rated to come under the intermediate zone of HBV prevalence ranging between 2-7%. There are 43 million estimated HBV carriers in India. Nearly 10% of them are highly infectious with multiplying virus. Nearly one third of the patients with acute hepatitis, two thirds of the cases with chronic liver diseases and hepatocellular carcinoma are due to HBV infection. Liver diseases due to HBV infection are considered to be the fourth or the fifth important cause of mortality in the most productive period of life (15 to 45 years). The disease is present as acute, chronic, and fulminant hepatitis as well as healthful carrier state (Mathew, 1997)

**Treatment strategies for Hepatitis B infection**

Till date, there is no effective treatment for hepatitis B infection and therefore prevention of this infection is considered an effective way to control the infection. The only treatment reported with variable success is the interferon therapy. Other promising treatment options for hepatitis B infection include nucleoside analogues such as lamivudine.

**Metabolic Disorders and Hepatic Damage**

Another feature of the liver that predisposes it to chemically induced liver injury is its ability to biotransform or metabolize chemicals. The important process is catalyzed by numerous enzymes which convert lipophilic compounds into more hydrophilic metabolites. These can be more readily excreted in the urine and the feces. The liver is the most important organ of biotransformation, due largely to its high content and large diversity of enzymes capable of metabolizing foreign as well as endogenous chemicals. These enzymes include UDP-glucuronyl
transferase, glutathione-S-transferase, cytochrome P450, FAD-containing mono oxygenase as well as others.

Toxic or reactive metabolites can initiate a series of events that ultimately result in liver injury. The initiation of lipid peroxidation, covalent modification of critical cellular molecules, consumption and ultimate depletion of important cellular components, mutations in DNA, and inhibition of protein synthesis (Hinson et al., 1994). At low rates of formation, reactive metabolites can be detoxified by conjugation with endogenous molecules or their damage can be repaired. However, if the rate of utilization of these endogenous molecules exceeds their synthesis, they will ultimately be depleted. At this stage, the hepatic parenchymal cell becomes extremely vulnerable to damage by reactive metabolites of chemicals.

**Manifestations of Hepatic Disfunctions**

*(Quiroga et al., 1992)*

**Hypoxia**

Owing to their active and complex metabolism, liver cells are readily injured by hypoxia, as in shock, venous congestion or anaemia. However, the dual blood supply to the liver affords some protection against hypoxic injury.

**Portal hypertension**

The portal hypertension is caused by obstruction to the blood flow through the liver. As a result, veins which provide an anastomosis between the portal and systems enlarge and some of the portal blood is shunted directly into the systemic circulation, instead of passing through the liver. This increases the blood level of toxic compounds absorbed from the gut, thus aggravating the effect of hepatocellular failure on the central nervous system and other organs.
Ascites

It is the increased sinusoidal pressure, as with severe inflammation or scarring of the liver that leads to the fluid accumulation in the abdomen that becomes more difficult to control with progressive decomposition.

Abnormal excretion

The accumulation of serum bilirubin in the serum, is normally taken up by the liver and excreted in to bile, resulting in jaundice.

Abnormal clearance

It is the decreased clearance of gut-absorbed proteins and ammonia from the liver which produces hepatic encephalopathy, a poisoning of brain with symptoms ranging from confusion to coma.

Liver cell necrosis and cirrhosis

Massive hepatic necrosis, which represents more severe degree of confluent necrosis is sequel to viral hepatitis or drug-induced injury. Clinically it results in fulminant acute hepatocellular failure.

In chronic liver disease piecemeal necrosis occurs and is defined as destruction of liver cells at an interface between parenchyma and fibrous tissue, together with a predominantly lymphocytic or plasma cell infiltrate.

Cirrhosis is the end point of many chronic liver diseases, since inflammation and cell death eventually yield to fibrosis or scar formation. Cirrhosis involves irreversible damage to the lobular architecture with diffuse fibrous bands of scar tissue surrounding nodules of regenerating hepatocytes (Sherlock and Summerfield, 1991).

Tumours

Primary tumours of the liver are relatively uncommon in developed countries though it is of great frequency in parts of Africa and the far east they are associated with cirrhosis. The liver is a very common site of
metastatic carcinoma, particularly from primary tumours, of the gastrointestinal tract (Mac Sween and Whaley, 1992).

**Hepatocellular carcinoma**

It is the primary malignant cancer of the liver and is associated with dismal prognosis. Chronic inflammatory disease of the liver may increase the risk of developing cancer. Nodular regeneration in cirrhotic livers may lead to cellular dysplasia, since errors are more likely to be made into more actively dividing cells (Redlich et. al., 1990).

**Hepatic failure**

Hepatocellular failure arises when total liver cell function falls below the minimum required to maintain a physiological state. It results from loss of a large number of liver cells and from impaired function of liver cells, attributable to interference with hepatic blood flow or interference with intracellular metabolic functions. Hepatic failure may be acute, as in massive liver cell necrosis due to hepatitis or drugs, or it may be chronic, for example in cirrhosis. The most important effects include, change in nitrogen metabolism with a rise in the blood level of toxic nitrogenous compounds, jaundice, defective synthesis of plasma proteins, hormonal and circulatory disturbances, functional renal failure (the hepatorenal syndrome), encephalopathy, coagulopathy, shock and sepsis (Kirsch et. al., 1995).

**Potentiation of chemically induced Hepatotoxicity**

It is well established that subjects exposed to several chemical agents simultaneously can exhibit altered pharmacologic or toxicologic responses. The effect of a second chemical can have a marked influence on the response elicited by a previously administered chemical and *vice versa*. Many of these have led to the discovery that biotransformation to a more active metabolite is involved in the hepatotoxic response. Many instances of potentiation of hepatotoxicity have been described. Individuals recovering from an acute ingestion of ethanol are more susceptible to the
liver damaging properties of the halogenated hydrocarbons than do individuals not ingesting ethanol. Cornish and Adefuin (1967) reported that the several aliphatic alcohols, such as methanol, ethanol, isopropanol, n-butanol, and tert-butanol exert similar potentiating effect on the acute inhalation toxicity of carbon tetrachloride.

Experimentally, it was shown that diabetes induced in rats by either alloxan or streptozotocin enhances the hepatotoxicity of carbon tetrachloride (Hanosono et al., 1975). Fiume (1972) found that an additional injection of endotoxin induces fulminant hepatitis within six hours, even in the NMRI mouse strain which is relatively resistant to D-galactosamine. Corresponding to this action of D-galactosamine, a-amanitin, an inhibitor of ribonucleic acid polymerase II, sensitized the liver to the action of a small dose of endotoxin leading to fulminant hepatitis.

**Morphological Responses of the Liver to Chemical Injury**

From a morphological aspect, the cells which comprise the liver can react to toxicant-induced damage in a limited number of ways (Kirsch et al., 1995). Therefore, there are only a few, rather well defined responses to a multitude of toxic chemicals. These responses are categorized as

(i) fatty change (or steatosis), which is a reversible condition
(ii) cell cytotoxicity which in the early stages (cell swelling) is reversible but can progress to irreversible coagulative necrosis
(iii) cholestasis, in which bile flow slows or ceases,
(iv) fibrosis and cirrosis, in which hepatocyte necrosis and deposition of collagen fibres disrupt normal hepatic architecture and function and
(v) The development of liver neoplasia.
D-Galactosamine/Lipopolysaccharide Model for the Induction of Hepatitis in Rats

"Galactosamine hepatitis" was a serendipitous observation made during studies of hepatic glycogen metabolism (Keppler et. al., 1968). After intraperitoneal injection to young adult rats of a rather large dose of D-galactosamine (D-GalN) hardly any glycogen could be found in the livers, while the organ looked conspicuously pale.

D-galactosamine is known as a constituent of glycoproteins and polysaccharides, but the existence of appreciable concentrations of free galactosamine in vivo has not yet been proved unequivocally. At any rate its toxic effects appear only when concentrations greatly exceeding physiological levels are maintained for several hours. Under these conditions the amino sugar or one of its derivatives might interfere independently with glycogen biosynthesis and with reactions leading ultimately to the morphologic changes observed (Keppler et. al., 1968).

Inspection of the liver of the rats intoxicated with D-galactosamine by light microscopy revealed morphological features closely resembling those seen in viral hepatitis, ie., acidophilic degeneration and appearance of so-called councilman bodies (indicative of apoptotic cell destruction), single cell necrosis and foci of hepatocellular necrosis, enlarged liver macrophages and preferentially periportal inflammatory infiltrations. Severe liver damage was also obvious from various serological data, for example, increased activities of hepatocellular enzymes, defects in export proteins such as lipoproteins, and disturbed fluxes of glutathione across the hepatocellular membrane (Irita et. al., 1994). These morphological alterations observed after administration of D-galactosamine could be found in the liver only; no other organ revealed inflammatory infiltration or cell necrosis. The specificity of D-galactosamine action was underscored by the failure of other structurally related sugars to elicit similar symptoms. An exception was the unphysiological 2-deoxy-D-galactose that provokes in guinea pigs the D-galactosamine specific pathological pattern.
Important factor for the elucidation of the molecular mechanisms leading to this hepatitis model was: the facile way to bring about the characteristic liver damage; the reproducibility of the biological, morphological and metabolic parameters; the control of commencement, severity, and the progress through choice of dose and time-course of galactosamine administration, and the clearly defined metabolism of this amino sugar.

**Bio Chemistry of D-Galactosamine**

**Metabolism, cell, organ and species specificity**

Galactosamine is preferentially taken up and metabolized by hepatocytes. Enzymes of galactose metabolism that are present in hepatocytes in relatively high activities catalyze phosphorylation, uridylation and 4-epimerisation of galactosamine.

GalN-1-phosphate, UDP-GalN, UDP-glucosamine, UDP-N-acetyl glucosamine, and UDP-N-acetyl galactosamine accumulate in hepatocytes after galactosamine administration. This leads to the depletion of the cells of uridine phosphates. After a galactosamine dose sufficient to provoke the hepatitis-like symptoms, the content of UDP-aminosugar derivatives in the liver exceeds by far the amount of all acid-soluble uracil nucleotides normally present in this organ.

This redistribution of uridylates cannot be compensated sufficiently by the normal liver, neither by an enhanced *de novo* synthesis of uridylate nor by uridylate synthesis through the salvage pathway. As a consequence, the cells run into deficit of important uracil nucleotides, particularly of UTP, UDP-glucose, UDP-galactose, and UDP-glucuronate (Decker and Keppler, 1972 and 1974). The exclusive action of galactosamine on hepatocytes is due to their ability to accumulate rapidly UDP derivatives of this aminosugar at the expense of other uridylates.

The typical pattern of pathological symptoms of galactosamine hepatitis could be provoked in all animal species investigated including
fish. Adult animals were more susceptible than new-born ones; in fact, liver damage could not be elicited prior to the third week of postpartum.

(Scheme of D-galactosamine metabolism and its connection with uridylate synthesis. Glc, Glucose; NAc, N-acetyl; UMP, Uridine 5'-monophosphate (uridylate).

The D-galactosamine-induced uridine triphosphate deficit and the consequences

D-galactosamine elicits a selective UTP deficit in the liver in vivo, in the isolated perfused liver, and in isolated hepatocytes in culture. The intracellular contents of CTP, ATP and GTP are not diminished under these conditions. A decrease of the UTP content in the rat liver to less than 10% of the normal value is obtained twenty minutes after injection of galactosamine.

The inhibition of transcription due to an UTP deficit was ascertained by measurements of guanosine incorporation into RNA. The imbalance in galactosamine-metabolizing hepatocytes between UDP-sugars and UDP-(N-acetyl) amino sugar has a profound influence on the synthesis of glycoconjugates. It is reflected in the inhibition of formation and secretion of serum glycoproteins (Gross et. al., 1990), of the synthesis of membrane glycoproteins, and of glycogen (Bachmann et.al., 1977). If the biosynthesis of some glycoproteins requires induces transcription, the simultaneous galactosamine-elicited UTP deficit blocks the formation of glycoproteins additionally at this level. This effect is particularly evident with acute-
D-Galactosamine

\[ \downarrow \]

Accumulation of UDP derivatives

\[ \downarrow \]

Deficit of UTP and UDP-hexoses

\[ \downarrow \]

Inhibition of RNA and glycoconjugate

\[ \downarrow \]

Sensitization towards TNF-α

\[ \downarrow \]

Apoptosis

\[ \downarrow \]

Inflammation of the liver

Effect of D-Galactosamine on hepatocyte.
phase proteins that would be induced under conditions of a beginning of galactosamine hepatitis.

The D-galactosamine-induced sensitization of the organism to endotoxin

The endotoxins are a family of lipopolysaccharides (LPS) derived from the outer cell membranes of gram-negative bacteria, for example Escherichia coli. Exposure to LPS commonly occurs in humans through many routes, including breathing bacteria-contaminated air or through translocation from the gut (Hewett and Roth, 1995).

These complex molecules penetrate from the gut into the portal blood following bacterial infections, sometimes even as the result of impaired intestinal resorption. LPS elicits the typical symptoms of inflammation (fever, pain and hypotension) and septic shock (Proctor, 1986). LPS is transported from the gut via the portal blood to the liver. There it encounters the liver macrophages (Kupffer cells) that reside in the sinusoidal lumen. Binding and partial endocytosis of LPS trigger the synthesis and secretion of a large number of signal molecules (mediators) including TNF-α, the interleukins (IL)-1β, and IL-6, the transforming growth factors-α and β (TGF-α and -β), the prostaglandins D2 and E2, thromboxane A2, and nitric oxide (NO) (Decker, 1990). These mediators find receptors on neighbouring cells including the hepatocytes and provoke specific responses in the target cells. Galactosamine does not increase the flux of LPS from the portal area to the liver. But an organism treated with galactosamine suffers a tremendous sensitization towards LPS (Galanos et al., 1979). It is most likely that the LPS effect is mediated to a considerable extent, if not fully by the TNF-α released from kupffer cells following contact with LPS (Lehmann et al., 1987).

The effect of TNF-α on the liver is manifold. Together with IL-8 and IL-1β it enhances the infiltration of granulocytes and their adherence to the sinusoidal endothelium (Schlayer et al., 1987). It also increases the
release of reactive oxygen intermediates, of nitric oxide, and of proteolytic enzymes. The activity of endogenous suppressors of cell death must also be considered in this context as they may be involved in the apoptosis of hepatocytes. The biochemistry of galactosamine explains the sensitization of hepatocytes towards apoptosis-and necrosis-inducing factors (Leist et al., 1994). Galactosamine is comparable to α-amanitin and actinomycin D as a sensitizing inhibitor of transcription. The effect of galactosamine differs from that of general inhibitors of transcription, such as actinomycin-D in that it is hepatocyte specific. This selectivity renders the synthesis of cytokines in neighbouring non-parenchymal cells unaffected. The increased supply of these apoptosis-inducing factors and the simultaneous suppression of hepatocellular transcription may explain the severity of the combinations TNF-α + GalN and LPS + GalN.

LPS is not a specific hepatotoxic agent. It causes severe cell damage in other organs as well. Although these cells do not metabolize galactosamine, and thus cannot suffer UTP deficiency, galactosamine nevertheless sensitizes these organs to the effects of LPS and TNF-α respectively. It is suggested that the hepatocytes secrete substances for example acute phase or TNF-α inhibitory proteins, that delay or attenuate the inflammatory effects (Libert et al., 1994). When the induced synthesis of these substances is suppressed by galactosamine, this protective mechanism can no longer operate.

**Signals from Damaged Hepatocytes to the Environment**

A D-galactosamine damaged hepatocyte is recognized by "professional phagocytes" (macrophages, neutrophilic granulocytes) in a similar way as a decaying erythrocyte. (i) Upon contact with the injured cell, these phagocytes are activated and release mediators that induce the process of apoptosis and necrosis, respectively, in the target cell (ii) the galactosamine-treated parenchymal cell itself is able to transmit information about its damage to the environment. This signal is recognized by non parenchymal cells and treated like an inflammatory agent.
(Gressner et al., 1995), (iii) the low level of TNF-α that is always present in the blood is insufficient to develop its apoptotic potential unless transcription is simultaneously inhibited in the target cells. Some hepatocytes may thus become damaged severely enough to trigger process (i) and (ii) respectively. These mechanisms are not mutually exclusive but might augment each other as the damage spreads within the organ.

"Galactosamine Hepatitis" As a Tool for Pharmacological Studies

Model of hepatotoxicity and hepatocellular apoptosis

Galactosamine hepatitis has become a much used model of liver damage and a useful tool in the investigation of interactions between the various cell types of the liver. It competes in this respect with older models such as carbon tetra chloride (CCl₄) hepatotoxicity. In the latter, changes being centrolobular, fatty infiltrations being the most conspicuous (Recknagel, 1983). The mechanism of action of CCl₄ and Galactosamine are quite different. With the former, the production of radicals and lipid peroxidation is the prominent feature. In galactosamine induced hepatotoxicity suppression of mRNA synthesis and partial inhibition of the post-translational completion of glycoconjugates predominates. As a secondary phenomenon, activation of macrophages and granulocytes, inflammatory processes, and apoptosis of hepatocytes may follow (Friedmann, 1993). Once the process has been started it progresses with necessity and leads to the "point of no return", irreversibly to apoptotic or necrotic cell death. Since it can be easily manipulated, galactosamine hepatitis serves as a useful model of hepatocellular apoptosis.

Use in studies of liver transplantation and hepatic encephalopathy

D-galactosamine hepatitis has been used in a large number of studies on the consequences, the prevention and treatment of acute liver failure. In neuropathology, galactosamine-induced fulminant hepatic failure was used to study the effect of hepatogenic, neurotoxic substances
on the permeability of the blood-brain barrier (Mc Chung et. al., 1990), the
neurochemical changes and histological alterations during the
development of encephalopathy (Zeneroli and Baraldi, 1990), the role of
glycosylation pattern for synaptic transmission, and the occupancy of the
benzodiazepine receptor during hepatic encephalopathy (Basile et. al.,
1990).

**Model for testing of anti-inflammatory, hepatoprotective and response modulating drugs**

Because of its specificity for hepatocytes galactosamine is very well suited and is widely used for investigations and pharmacokinetic studies of hepatoprotective, shock-preventing, and therapeutic effectors in inflammatory processes of the liver (Kikuchi et. al., 1994). Furthermore, galactosamine-induced liver injury served as a model for testing and elucidating the protective and even therapeutic value of flavones and quinones, that are known as antioxidants and of other plant products that are claimed to be hepatoprotective (Hoffman-Bohm et. al., 1992 and Nagakawa et. al., 1993).

The model “Galactosamine hepatitis” has not lost its actuality and usefulness twenty five years after its discovery. New methodological and conceptual approaches allowed fresh insights into the pathophysiology of the liver and its influence on other organs. The case with which this hepatocellular injury can be handled experimentally recommends it also for therapeutic studies in animal models.

**Spices**

According to the International organization for Standardization (ISO) there is no marked distinction between spices and condiments. The term spice is used for aromatic plant products or mixtures thereof, either as whole or in ground form. It is rather loosely applied to an assortment of dried barks, roots, seeds fruits and flower parts. Spices which impart
aroma, flavour and piquancy to food are generally tropical in origin (Samba Murty and Subrahmanyam, 1989).

Samba Murty and Subrahmanyam (1989) noticed that condiments on the other hand are spices that are usually added to food after cooking. In contrast, when the aromatic vegetable product comes from a temperate plant it is considered as culinary herb (non-woody) as in case of bay leaves, coriander, fennel, mustard etc.

Herbs make all the difference to food; the cuisine of a region is characterized as much by the herb it uses as by the staple foods. Fragrant mixtures have come to characterize the cooking of certain regions—'bouquet garni' in France; 'garam masala' in the Northern India and five-spice powder throughout China and Vietnam are very popular. Mint, an uncompromising flavour, affects the taste buds very differently in Moroccan mint tea, mint sauce, mint julep, harissa (a Tunisian paste made from mint, chillies, cumin, coriander, caraway seeds and garlic), tabbouleh and tzatziki (a mint, cucumber and yogurt dip). Thus some herbs and spices are almost universally popular. Pepper, ginger, cinnamon, nutmeg, cloves and garlic are the beloved of most cuisines (Bown, 1995).

**History of Spices**

The history of spices' is one of the spiciest chapters in the history of the plant kingdom. Historically, spices have been responsible for the rise and fall of empires and the great sea voyages to explore the distant corners of the globe. In fact, spices have played an important role in shaping the course of history; they have been connected with adventure, conquest, exploration etc., around the world. In the later half of the fifteenth century, both Portugal and Spain explored sea routes to the Spice Islands (Moluccas). Christopher Columbus sailed west from Spain in 1492, hoping to reach the Spice Islands ahead of the Portuguese, but he failed in his primary mission. Instead, he discovered America and also helped in the
discovery of two of the three important New World spices, all spice (*Pimenta officinalis*) and red pepper (*Capsicum* spp). The third important New World Spice is vanilla (*Vanilla planifolia*). In the early part of the eighteenth century spices were smuggled away and planted around the world, especially in the West Indies. Nowadays, substantial plantations are grown in America. However, the vast majority of spices are still obtained from the wetter parts of the tropics, chiefly Asia (Pruthi, 1992).

**Intake of Spices**

Spices and condiments have played a prominent part in all the civilization of antiquity, in ancient India and China. They were among the first objects of commerce between the East and the West. Population-wise average dietary intake of common spices has been estimated at 0.5g/person per day in Europe and 1.0g/person per day in New Zealand. According to the American Spice Trade Association, per capita spice consumption in the United States was ~4g/person per day (3.6lb/person per year), and hot spices such as black and white pepper, red pepper, and mustard seed account for 41% of US spice usage. In contrast, in the Indian subcontinent, turmeric consumption alone has been estimated at 1.5g/person per day. Generally, cuisines that traditionally do not include much meat use a wider variety of spices for seasoning. These include cuisines in areas of the world where vegetarianism has existed for centuries, such as among followers of Hinduism and Buddhism. Nearly all spices important in cooking today are of Asian origin, with the exception of all spices, vanilla and chilli. Thus, globally, the amounts and types of spices used vary widely (Pruthi, 1992).

**Beneficial Effects of Spices**

Medicinal meals remain important in traditional Chinese and Ayurvedic practice. In the Siddha system practised in Tamil Nadu, every meal must contain some forms of medicinal ingredients too in order to avoid the consumption of medicines separately. Plants have the capacity to synthesize a diverse array of chemicals, and understanding how
phytochemicals function in plants may further our understanding of the mechanism by which they benefit humans.

In warm countries, the effects of eating hot spices, such as chilli, ginger and pepper, are to raise the metabolic rate, increasing perspiration, effectively cooling the body, and speeding the excretion of toxins. Spices act also as preservatives, of great importance in warm regions where food deteriorates rapidly (Bown, 1995).

Herbs add colours as well as flavour. Soups made from pale ingredients are much more appetizing when flecked with finely chopped parsely or chives. Yellow is an especially appetizing colour-most subtle in saffron, and the brightest in turmeric. Though seldom seen in the form of seeds, annatto is a colouring appreciated every day in butter, margarine, and “red” cheeses, which would otherwise be cream-coloured. Paprika gives a glorious brick-red colour to dishes such as goulash. It is made from dried, powdered red peppers (Capsicum annuum), as is cayenne, but can be used in much larger quantities (Bown, 1995).

Peppermint has a soothing, mildly anaesthetic effect on the digestive tract, hence the popularity of after dinner mints and peppermint tea. Perilla, which is used with raw fish dishes in Japan, contains antidotes to seafood poisoning. Garlic is an excellent gastric disinfectant, well worth taking in capsule form, as well as in food, while travelling to prevent bouts of diarrhoe and vomiting. The therapeutic side to culinary herbs was once more popular than it is today (Bown, 1995).

**Chemical Properties of Spices and Their Significance**

Essential oils are the key components in both the flavour and beneficial effects of herbs and spices, and many are strongly antiseptic, protecting against harmful microorganisms. Fennel, dill and caraway contain carminative oils that almost instantly relieve gas. They are particularly good with foods that many people find indigestible – fennel
with oily fish, dill with cucumbers, and caraway with coleslaw or rich meats (Bown, 1995).

Herbs and spices will also increase the vitamin and mineral content of the food, and improve digestion. Garlic is rich in germanium, which has beneficial effects on the circulation. The bitter element in herbs and spices serves to prime the digestive system, stimulating the liver and gall bladder, improving digestion, especially of fats, and helping the elimination of toxins. Aperitifs with a hint of bitterness, and raw foods such as salads and 'cruditès', are traditionally eaten for this purpose (Bown, 1995).

In plants, these compounds function to attract beneficial and repel harmful organisms, serve as photoprotectants and response to environmental changes. In humans, they can have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial effects. Embracing a cuisine rich in spice, as well as in fruit and vegetables, may further enhance the chemopreventive capacity of one's diet (Lampe, 2003).

**Phytochemistry of Spices**

Over millions of years, plants have developed the capacity to synthesize a diverse array of chemicals. In generally, these phytochemicals function to attract beneficial and repel harmful organisms, serve as photoprotectants, and respond to environmental changes. For example, numerous classes of phytochemicals, including the isoflavones, anthocyanins, and flavanoids, function as phytoalexins, substances that assist a plant to resist pathogens. Various glycosides of these also render plants unpalatable and thereby reduce intake by animals. Paradoxically, carotenoids aid in light collection under conditions of low light or help to dissipate excess absorbed energy as heat under conditions of high sun exposure; most plants have the flexibility to alter their carotenoid composition in response to growth under deep shade or full sunlight.
(Demmig-Adams et al., 1996). Understanding how phytochemicals function in plants may further our understanding of mechanisms by which they may benefit humans. Paradoxically, spices are grown as food enhancers, despite the intention of these tasty constituents to discourage consumption of the plant (Lampe, 2003).

Only a small number of primary compounds serve as precursors of the large array of phytochemicals produced by higher plants, and most of these are obtained from the early products of photosynthesis (Conn, 1995). The constituents responsible for the flavor properties of spices are products of secondary metabolism in plants: that is, they are not vital for plant tissue synthesis or energy production and storage, but their production is essential to the viability of the plant. Most known phytochemicals arise from 3 well-recognized metabolic pathways: the shikimate pathway, the cinnamic acid pathway, and the isoprenoid pathway. The shikimate pathway is a major source of carbon for many compounds in part because the 3 aromatic amino acids formed as end products: phenylalanine, tryptophen, and tyrosine, are important precursors that feed into several synthetic pathways. The phenylalanine is the starting material for the cinnamic acid pathway, which produces numerous phenolic acids, coumarins, flavonoids, isoflavonoids, and lignans. These 3 amino acids, in addition to others, also provide the carbon atoms for production of glucosinolates in Brassicaceae. Pyruvic acid, an early product of photosynthesis, is the starting point for the isoprenoid pathway, from which families of carotenoids, terpenes, saponins, and so forth are derived.

Despite the large number of phytochemical classes, most plants contain only a few of them, and botanically related plants often contain similar or even the same constituents. As a result, spices tend to cluster in certain plant families, while other families do not contain any aromatic plants (Katzer, 2002). The terpenes and terpene derivatives are probably the most important class of aroma compounds, with monoterpines contributing to the fragrance of 90% of spices (Katzer, 2002).
Monoterpenes occur in many different plants, and the characteristic aroma of a spice results from a specific mixture of monoterpenes and not a specific compound (Katzer, 2002).

Many phytochemicals are present in plants as glycosides (i.e., with a sugar moiety attached). Generally, glycosides are non-volatile and lack fragrance. Cleaving the glycosidic bond yields the aglycon, which itself may be volatile and fragrant. For example, glucosinolates of the cabbage family (Brassicaceae) are hydrolysed by the plant enzyme thioglucosidase (myrosinase) when cells are damaged (e.g. cut or chewed), yielding the pungent isothiocyanates, and vanillin is released from a glycoside precursor during drying, a step in the processing of vanilla beans. Thus, how a spice is processed will also determine the amount and form of its constituents (Lampe, 2003).

**Nigella Sativa**

- **Common name** - Black Cumin (or) Black seed
- **Botanical name** - *Nigella sativa* (L)
- **Family** - Ranunculaceae
- **In India** - Kalonji

**Botanical and Historical Background**

Among the promising medicinal plants, *Nigella sativa* a dicotyledon of the *Ranunculaceae* family is an amazing herb with a rich historical and religious background (Goreja, 2003). *N. sativa* is believed to be indigenous to the Mediterranean region but has been cultivated into other parts of the world including the Arabian Peninsula, Northern Africa and parts of Asia. It was brought to Asia by physicians and cultivated in India. Now-a-days it is cultivated throughout India, whereas it is widely grown too. It is a busy, self-branching plant not more than 3mm in length with white or pale to dark blue flowers. It is in flower in July and the seeds ripen in September. 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
Plate - I

*Nigella sativa* L.
and petioled and the upper leaves are long (6-10 Cm) the stalk of the plant reaches a height of 12-18 inches as its fruit, the black seed, matures. *N. sativa* is bisexual, reproduces with itself and forms a fruit capsule which consists of many white trigonal seeds. When the fruit capsule has matured, it opens up and the seeds contained within are exposed to the air, becoming black in colour. The seed is aromatic with a nutmeg scent (Sehleicher and Saleh, 1998).

The seeds of *N. sativa* are the source of the active ingredients of this plant. It is the black seed referred to by the prophet Mohamed (peace be upon him) as having healing powers as “Use this black seed, it has a cure for every disease except death” (Goreja, 2003). Black seed is also identified as the curative black cumin in the Holy Bible and is described as the Melanthion of Hippocrates and Discroides and as the Gith of Pliny (Junemann, 1998).

**Soil, Climate and Propagation**

Easily grown in any good garden soil, preferring a sunny position. Prefers a light soil in a warm position. Seed - sow spring or early autumn in situ. The autumn sowing might not be successful in harsh winters. Plants can be transplanted if necessary.

**Constituents**

By HPLC analysis of *N. sativa* oil, thymoquinone (TQ), dithymquinone (DTQ), which is believed to be nigellone, thymohydroquinone (THQ), and thymol (THY), are considered the main active ingredients [Omar *et al.*, 1999].
Chemical structure of the active ingredients; TQ, DTQ, THY and THQ, in the oil of *N. sativa* L seed.

*N. sativa* seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids [Omar et al., 1999 Al-jassir, 1992 Bhatia and Bajaj, 1972 Chun et al., 2002 and Correa et al., 1986]. Fractionation of whole *N. sativa* seeds using SDS-PAGE shows a number of protein bands ranging from 94 to 10 kDa molecular mass [Haq et al., 1999]. Monosaccharides in the form of glucose, rhamnose, xylose, and arabinose, are also found. *N. sativa* seeds are rich in the unsaturated and essential fatty acids. Chemical characteristics, as well as fatty acid profile of the total lipids, revealed that the major unsaturated fatty acid is linoleic acid, followed by oleic acid [Omar et al., 1999 Al-jassir, 1992 Mahmoud et al., 2002 Nickavar et al., 2003 and Ramadan, 2002]. The major separate individual phospholipids classes is phosphatidylcholine, followed by phosphatidylethanolamine, phosphatidylserine, and phosphatidylinisitol, respectively [Omar et al., 1999, Al-Jassir, 1992 and El-Mahmoudy et al., 2002]. The seeds contain carotene which is converted by the liver to vitamin A [Al-jassir, 1992]. The
Chemical composition of *Nigella sativa* L. Seed

<table>
<thead>
<tr>
<th>Group</th>
<th>Sub-group</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed oil</td>
<td>Unsaturated fatty acids</td>
<td>Arachidonic eicosadienoic, linolenic, oleic and almitoleic acid</td>
</tr>
<tr>
<td>(32 – 40%)</td>
<td>Saturated fatty acids</td>
<td>Palmitic, stearic and myristic acid</td>
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<tr>
<td></td>
<td></td>
<td>Beta-sitosterol, cycloecalenol, cycloartenol, sterol esters and sterol</td>
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<td></td>
<td></td>
<td>glucosides</td>
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<tr>
<td>Volatile oil</td>
<td></td>
<td>Nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, cardarcol,</td>
</tr>
<tr>
<td>(0.4-0.45%)</td>
<td></td>
<td>α &amp; β-pinene, d-limonene, d-citronellol, p-cymene and 2-(2-methoxypropyl)-5-</td>
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<tr>
<td></td>
<td></td>
<td>methyl-1,4-benzenediol</td>
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<tr>
<td>Proteins</td>
<td>Aminoacids</td>
<td>Arginine, glutamic acid, leucine, lysine, methionine, thyrosine, proline and</td>
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<td>(16-19.9%)</td>
<td></td>
<td>threonine, etc.</td>
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<tr>
<td>Alkaloids</td>
<td></td>
<td>Nigellicine, nigellidine, nigellimine-N-oxide</td>
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<tr>
<td>Coumarins</td>
<td></td>
<td>6-methoxy-coumarin, 7-hydroxy-coumarin, 7-oxycoumarin</td>
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<tr>
<td>Saponins</td>
<td>Triterpenes</td>
<td>α-hedrin</td>
</tr>
<tr>
<td>Steroidal</td>
<td></td>
<td>Steryl-glucosides, acetyl-steryl-glucoside</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td>Calcium, phosphorous, potassium, sodium and iron</td>
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<tr>
<td>(1.79-3.74%)</td>
<td></td>
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<tr>
<td>Carbohydrates</td>
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<tr>
<td>(33.9%)</td>
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<tr>
<td>Fiber</td>
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<tr>
<td>(5.5%)</td>
<td></td>
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<tr>
<td>Water</td>
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<td>(6%)</td>
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</table>
N. sativa seeds are also a source of calcium, iron, and potassium [Al-Gaby, 1998].

**Immunopharmacological Properties of N. Sativa Seeds**

Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and in Asian countries for the promotion of good health and treatment of many ailments including fever, common cold, headache, asthma, rheumatic diseases, and various microbial infections and to expel worms from the intestines. It is also used for scorpion and spider stings and bites of snake, cat and dog. In addition, it is used as a flavoring additive to bread and pickles [El-Kadi and Kandil, 1986 and Al.Jishi, 2000]. The multiple use of *N. sativa* in the folk medicine encouraged many investigators to isolate the possible active components and to conduct in vivo and in vitro studies on laboratory animals and human beings in order to understand its pharmacological actions. These include, immune stimulation [El-Kadi and Kandil, 1986 and Al.Jishi, 2000], anti-inflammatory [Houghton et al., 1995], anti-cancer [Salomi et al., 1991], anti-microbial [Topozada et al., 1965 and El-Fatatry, 1975], anti-parasitic [Akhtar and Riffat, 1991], anti-oxidant [Nair et al., 1991 and Badary et al., 2000] and hypoglycemic effects [Al-Awadi and Gumma, 1987 and Bamosa et al., 1997].

**Anti-Oxidant Properties**

**Oxidant stress system and toxicity**

Oxidative damage to biological structures has been implicated in the toxicity-induced pathophysiology of several diseases, in particular cardiovascular disease and cancer [Maxwell, 1999]. The cause of this oxidative damage has been reported to be the shift in the balance of the pro-oxidant (free radicals) and the anti-oxidant (scavenging) mediators, where pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress, or due to the poor scavenging capability in the body [Hogg, 1998]. Free oxygen radicals, including O₂, OH, and NO (collectively known as oxidative stress),
are electrically charged molecules that attack cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins and enzymes present in the body [Hogg, 1998]. The attacks by ROS cause damage to cell structure and function and can eventually destroy them. ROS are produced mainly by certain cells of immune system including macrophages (MΦ) and neutrophils [Bandhopabhyay, 1999]. It has recently reported that suppression of immune cell function associated with chemotherapy [Angulo et. al, 2000], radiotherapy [Billiau et. al., 2003], infection [Abrahamsohn and Coffman, 1995 and Goni et. al, 2002] and in tumor-bearing hosts [Kusmartsev et. al, 2000] is mediated by production of NO produced by immature myeloid cells that are massively generated under these conditions [Angulo et. al., 2000a Mazzoni et. al., 2002 and Dupuis et. al, 2003]. The central role of ROS in mediating the pathology in several diseases has stimulated interest in the possible role of natural anti-oxidant agents in preventing the development of these diseases. It has been reported that the health promotive, disease preventive and rejuvenation approach based on using medicinal plants in “Ayurveda,” an ancient Indian systems, is due to the anti-oxidant effects of these plants [Govindarajan et. al, 2005]. One of the potential properties of N.sativa seeds is the ability of one or more of its constituents to reduce toxicity due to its anti-oxidant activities. Of the studies that have been performed to evaluate the different effects of N.sativa, majority (more than 35) of the studies have confined to address its antitoxic properties both in vitro and in vivo.

**In vitro anti-oxidant activities**

In vitro studies show that N.sativa seed extract induces inhibition of the hemolytic activities of snake and scorpion venoms [Sallal et. al., 1996], protects erythrocytes against lipid peroxidation, protein degradation, loss of deformability, and increased osmotic fragility caused by H2O2 [Suboh et. al., 2004]; and protects laryngeal carcinoma cells, from programmed cell death (apoptosis) induced by lipopolysaccharide (LPS) or cortisol [Corder, 2003]. These results indicate to the antitoxic effects of N.sativa seed.
components that could be attributed to its anti-oxidant properties. Several in vitro studies confirm this hypothesis. For instance, essential oil obtained from six different extracts of *N. sativa* seeds and from a commercial fixed oil showed anti-oxidant effects with almost identical qualitative effects. Differences, however, were mainly restricted to the quantitative composition [Burits and Bucar, 2000]. The crude *N. sativa* oil and its fractions (neutral lipids, glycolipids, and phospholipids) showed potent in vitro radical scavenging activity that is correlated with their total content of polyunsaturated fatty acids, unsaponifiables, and phospholipids, as well as the initial peroxide values of crude oils [Ramadan et al., 2003]. Moreover, preincubation of peritoneal MΦ with aqueous extract or the boiled fraction of the extract of *N. sativa* seeds caused a dose-dependent decrease in NO production when activated with LPS of E. coli [Mahmood, 2003]. Interestingly, TQ and a synthetic structurally-related tert-butylhydroquinone, also efficiently inhibited iron-dependent microsomal lipid peroxidation in vitro in a concentration-dependent manner [Badary et al., 2003]. TQ also induced significant protection of isolated hepatocytes against tert-butyl hydroperoxide induced toxicity evidenced by decreased leakage of ALT and AP [Daba and Abdel Rahman, 1998]. In addition TQ in a dose-and time-dependently manner, reduced nitrite production, a parameter for NO synthesis, and decreased both gene expression and protein synthesis levels of iNOS in supernatants of LPS-stimulated MΦ without affecting the cell-viability [El-Mohmoudy et al., 2002]. Stimulation of polymorphonuclear leukocytes with TQ showed protective action against superoxide anion radical either generated photochemically, biochemically, or derived from calcium ionophore, indicating to its potent superoxide radical scavenger [Nagi and Mansour, 2000].

**In vivo anti-oxidant activities**

Both hepatotoxicity and nephrotoxicity are associated with alteration in the levels and activities of certain mediators such as L-alanine aminotransferase (ALT), alkaline phosphatase (AP), lipid peroxide (LPD), and the oxidant scavenger enzyme system including, glu-tathione (GSH)
and superoxide dismutase (SOD). The anti-oxidant effects of *N. sativa* have been examined using different hepatic and kidney toxicity *in vivo* murine models induced by tert-butyl hydroperoxide, carbon tetrachloride (CCl4), doxorubicin (DOX), gentamicin, mehtionine, potassium bromate (KBrO3), cisplatin, or *schistosoma mansoni* infection.

**In vivo anti-oxidant activities of *N. sativa* seed oil**

In CCl₄-induced toxicity, *N. sativa* oil protected against hepatotoxicity coinciding with improvement in serum lipid profile [El-Dakhakhny et al., 2000 and Nagi et al., 1999], decreasing the elevated serum K and Ca levels, ameliorating the reduced RBC, WBC, PCV, and Hb levels [Meral and Kenter, 2003 and Merral et al., 2001], decreasing the elevated LPD and liver enzyme levels, and increasing the reduced anti-oxidant enzyme levels [Kanter et al., 2003]. Moreover, treatment with *N. sativa* oil prevented CCl₄-induced liver fibrosis in rabbits with improvement of the anti-oxidant status [Turkdogan et al., 2001]. In gentamicine-induced toxicity, treatment with *N. sativa* oil produced a dose-dependent amelioration of the biochemical and histological indices of nephrotoxicity, coincided with the increase in the scavenger defense system, including GSH concentration and the total anti-oxidant status in renal cortex [Ali, 2004]. In KBrO₃-mediated renal oxidative stress prophylaxis of rats orally with *N. sativa* extract resulted in a significant decrease in renal LPD and oxidative stress that coincided with marked recovery of renal glutathione content and anti-oxidant enzymes [Khan et al., 2003]. Using gastric ulcer model induced in rats by oral administration of ethanol that causes a significant reduction in free acidity and glutathione level, pretreatment of rats with *N. sativa* before induction of ulcer induced a significant increase in glutathione level, mucin content, and free acidity with a protection ratio of 53.56% as compared to the ethanol group [El-Dakhakhny et al., 2000]. Taken together, these findings show the potential antitoxic effect of *N. sativa* seeds in form of crude extracts or oil mediated by their anti-oxidant properties.
**In vivo anti-oxidant activities of TQ**

Pro-phylactic treatment of mice with TQ 1 h before CCl₄ injection ameliorated hepatotoxicity of CCl₄ as evidenced by the significant reduction of the elevated levels of serum enzymes, and significant increase of the hepatic GSH content [Ramadan et. al., 2003 Ali, 2004 and Enomoto, 2001]. Treatment of mice with the other volatile oil constituents, p-cymene or alpha-pinene, however, did not induce any changes. The effect of TQ on the nephrotoxicity, cardiotoxicity, and oxidative stress induced by DOX in rats shows that its administration counteracted the development of nephrotic hyperlipidemia, and hyperproteinuria; and restored the biomarker’s values of oxidative stress towards normal [Badary et. al., 2000].

The pathogenesis in hyper homocysteinemia (HHcy), including gastric lesion, liver fibrosis, and cardiotoxicity, is known to be linked with free radical formation associated with higher risks of coronary, cerebral and peripheral vascular disease. Interestingly, oral pretreatment of rats with either crude *N.sativa* oil or TQ protected against methionine-induced HHcy through amelioration of the plasma levels of triglycerides, lipid peroxidation, cholesterol, and in the activities of the anti-oxidant status [El-Saleh et. al., 2004]. Also, when rats were subjected to ischaemia/reperfusion, injection of *N.sativa* oil or TQ tended to normalize the level of LDH, GSH, and SOD; TQ showed higher effect than that induced by the oil [El-Abhar et. al., 2003]. Fanconi Syndrome (FS), induced by ifosfamide, is characterized by wasting of glucose, electrolytes and organic acids, along with elevated serum creatinine and urea, as well as decreased creatinine clearance rate. Administration of TQ with the drinking water to rats before and during ifosfamide treatment ameliorated the severity of ifosfamide-induced renal damage and improved most of the alterations of biochemical parameters [Badary, 1999], including renal GSH depletion and LPD accumulation.
Schistosoma mansoni infection induces marked alteration in the liver function due to the heavy worm and egg burden deposited in the liver. Administration of N.sativa oil markedly reduced the worm and egg burden, coincided with partial amelioration of the schistosoma-induced liver fibrosis and changes in ALT, GSH, AP activities in serum [Mahmoud et al., 2002], suggesting that the anti-schistosomal effect of N.sativa oil might be induced partly by its anti-oxidant effect. Similarly, treatment with N.sativa oil decreased the hepatocellular necrosis, degeneration and advanced fibrosis in CCl4-induced liver fibrosis in rabbits [Turkdogan et al., 2001]. S. mansoni infection also induces a genotoxic effect, causing a significant increase in the incidence of chromosomal aberrations [Hamada et al., 1992]. Interestingly, treatment of S. mansoni-infected mice with N.sativa oil or purified TQ induced a protective effect on the infection-induced genotoxicity evidenced by reduction in the percentage of chromosomal aberrations and the incidence of chromosome deletions and tetraploidy [Aboul-Ela, 2002].

Coupling the fact that N.sativa seeds have been used in folk medicine with its antitoxic findings discussed above, it is apparent that the crude oil of N.sativa oil and its active constituents can lower oxidative stress-mediated toxicity induced accidentally by environmental or infectious factors, or by anti-cancer drugs. For instance, chemotherapy, cyclophosphamide and other anti-cancer drugs, is currently used in pre-clinical and clinical studies either as anti-cancer therapy or in combination with cancer immunotherapy [Dudley et al., 2005]. Since chemotherapy induces massive expansion of the immature granulocytes, which produce large amount of NO, it might be feasible to follow chemotherapy with TQ treatment that might alleviate the suppressive effects on the immune responses by chemotherapy-induced NO.

**Anti-histaminic properties**

Histamine is released by body tissues, creating allergic reactions associated with conditions such as bronchial asthma. There is an
indication from the traditional use of *N.sativa* seeds that its active ingredients have a substantial impact on the inflammatory diseases mediated by histamine. It was found from four decades that DTQ dimmer isolated from *N.sativa* seed's volatile oil, under the name of 'Nigellone,' when given by mouth to some patients suffering from bronchial asthma, it suppressed symptoms in the majority of patients [El-Dakhakhny, 1965]. In this study, nigellone was administered to children and adults in the treatment of bronchial asthma with effective results and with no sign of toxicity. In a clinical study, treatment of patients with allergic diseases, including allergic rhinitis, bronchial asthma, atopic eczema, with *N.sativa* oil decreased the IgE, and eosinophil count, endogenous cortisol in plasma and urine [Kalus et. al, 2003], indicating the effectiveness of *N.sativa* oil as adjuvant for the treatment of allergic diseases.

Indeed, the anti-allergic effect of *N.sativa* seed components could be attributed to its anti-histaminic effects. *In vitro* studies support this notion. Aqueous extract of *N.sativa* has shown relaxant and anti-histaminic effects on precontracted guinea pig tracheal chains. This effect was observed in the presence of both ordinary and calcium free Krebs solution, but with no effect in the absence of KCl induced contraction, suggesting that the calcium channel blocking effect of this plant does not contribute to its relaxant effect [Boskabady et. al, 2004]. In addition, the potent inhibitory effect of nigellone on histamine release from rat peritoneal mast cells, stimulated by different secretagogues; antigen sensitized cells, compound 48/80 and the Ca-ionophore A23187, was found to be mediated by decreasing intracellular calcium by inhibition of protein kinase C, a substance known to trigger the release of histamine [Gilani et. al, 2001 and Chakravarty, 1993]. Moreover, by investigating its effect on the guinea pig isolated tracheal zig-zag preparation, TQ caused a concentration-dependent decrease in the tension of the tracheal smooth muscle precontracted by carbachol [Al-Majed et. al, 2001]. Moreover, TQ totally abolished the pressor effects of histamine and serotonin on the guinea pig isolated tracheal and ileum smooth muscles. These effects of
were suggested to be mediated, at least in part, by inhibition of lipoxygenase products of arachidonic acid metabolism and possibly by non-selective blocking of the histamine and serotonin receptors [Al-Majed et al., 2001].

Preclinical and clinical studies have also shown anti-histaminic effects for *N. sativa* seeds. Using gastric ulcer model induced by oral administration of ethanol, which caused a significant increase in mucosal histamine content, rat pretreated with *N. sativa* oil before induction of ulcer induced a significant decrease in gastric mucosal histamine content with a protection ratio of 53.56% as compared to the ethanol group [El-Dakhakhny et al., 2000]. In contrast to the relaxant effect observed above for TQ, another study showed a stimulant effect. In this study, the effect of the volatile oil of *N. sativa* on the respiratory system of the urethane-anaesthetized guinea-pig was compared to those of TQ [El-Tahir et al., 1993]. Both the respiratory rate and the intratracheal pressure were increased, in a dose-dependent manner, by the i.v. administration of the oil mediated via release of histamine with direct involvement of histaminergic mechanisms and indirect activation of muscarinic cholinergic mechanisms [El-Tahir et al., 1993]. On the other hand, i.v. administration of TQ induced significant increases in the intratracheal pressure without any effect in the respiratory rate. Taken together, it seems that different active ingredients of *N. sativa* oil possess different impacts on the histamine release. The active ingredient nigellone of the crude extract of *N. sativa* seeds acts as calcium channel blocker(s), which might explain the beneficial traditional therapeutic uses of *N. sativa* toward diarrhea, asthma and hypertension.

**Anti-inflammatory properties**

**Inflammatory mediators**

Progression and persistence of acute or chronic state of inflammation are mediated by a number of mediators, including eicosinoids, oxidants, cytokine, and lytic enzymes secreted by the
inflammatory cells macrophages and neutrophils [Lefkowitz et al., 1999]. As discussed earlier ROS, in particular NO, initiates a wide range of toxic oxidative reactions causing tissue injury. In addition to the ROS-induced inflammation, inflammation is also mediated by two main enzymes: cyclooxygenase (COX) and lipoxygenase (LO) [Williams et al., 1999]. COX yields from arachidonic acid prostaglandins (PGE) and thromboxane [VanRyn et al., 2000], while LO catalysis the formation of leukotriens (LT). Both PGE and LT function as the main mediators of allergies and inflammation.

**In vitro anti-inflammatory effects of N. sativa seed components**

Several in vitro studies reproducibly reported the inhibitory effects of *N. sativa* oil and its active ingredients on the production of these mediators. For instance, TQ and the crude fixed oil of *N. sativa* inhibited both COX and 5-LO pathways of arachidonate metabolism in rat peritoneal leukocytes stimulated with calcium ionophore A23187, as shown by dose-dependent inhibition of thromboxane B2, LTC4 and LTB4, respectively; TQ showed higher effects [Mansour and Tornhamre, 2004 and Houghton et al., 1995]. Both substances also inhibited non-enzymatic peroxidation in brain phospholipid liposomes; again TQ was about ten times more potent. Interestingly, however, the inhibitory effect of the fixed oil of *N. sativa* on eicosanoid generation and lipid peroxidation was greater than that of TQ, suggesting that other components, such as unsaturated fatty acids, may contribute also to the anti-eicosanoid and anti-oxidant activities of *N. sativa* oil.

Furthermore, in vitro treatment of calcium- or ionophore-stimulated polymorphonuclear leukocytes (neutrophils) with either crude extract of *N. sativa*, nigellone, or TQ produced a concentration dependent inhibition of 5-LO products and 5-hydroxy-eicosa-tetra-enoic acid production [El-Dakhakhny et al., 2002]. Thus, inhibition of both COX and 5-LO pathways is key factors mediating the anti-inflammatory effects of the crude oil of *N. sativa* and its active ingredients.
In vivo anti-inflammatory effects of *N.sativa* seed components

Components of *N.sativa* have also been shown appreciated anti-inflammatory effects in several inflammatory diseases, including experimental allergic encephalomyelitis (EAE), colitis, and arthritis. EAE is an autoimmune demyelinating disease of the central nervous system that is widely accepted as an animal model for the human multiple sclerosis that is mediated by T-cells, while oxidative stress also plays a central role in the onset and progression of this disease [Chakrabarty et al., 2003]. When EAE animal received TQ, they showed higher glutathione level, no perivascular inflammation with no disease symptoms, compared with EAE untreated animals. These data reveal the therapeutic potential of TQ in EAE model [Mohamed et al., 2003] and indicate to its possible efficacy in treatment of multiple sclerosis in humans.

Ulcerative colitis is another inflammatory disease that is characterized by cycles of acute inflammation, ulceration and bleeding of the colonic mucosa. Although the pathogenesis of colitis remains poorly understood, various mediators, such as eicosanoids, leukotrienes, platelet activating factor and oxygen-derived free radicals have been implicated in the pathogenesis of this disease [Nieto et al., 2000]. Treatment with anti-inflammatory [Campieri et al., 1991 Gionchetti et al., 1991] or antioxidant agents has been shown to ameliorate the disease symptoms [Koch et al., 2000 and Choudhary et al., 2001].

In a recent study, the effects of TQ on the acetic acid-induced colitis in rats by intracolonic injection of 3% acetic acid showed that pretreatment of animals for 3 days with TQ led to complete protection against acetic acid-induced colitis with a comparable or even higher effects than sulfasalazine, an anti-colitis drug [Mahgoub, 2003]. The anti-colitis effects of TQ were associated with reversed biochemical and histopathological changes towards the normal. This study suggested that the anti-colitis effect of TQ is due to its anti-oxidant and anti-histaminic activities.
It has been observed for a long time that the *N. sativa* oil has an anti-inflammatory effect relieving the effects of arthritis [Ali and Blunden, 2003]. Consistent with these observations, recent studies have reported also that externally in an ointment form, the anti-inflammatory activity of the black seed was found to be the same range as that of other similar commercial products without induction of skin allergy [Zedlitz et al., 2002]. Injection of emulsion of *N. sativa* oil induced significant reduction in endotoxin shock in response to LPS [Hirschberg et al., 1990] and did markedly inhibit oedema induced by carrageenan or croton oil [Hajhashemi et al., 2004]. Similar to the anti-inflammatory effects of *N. sativa* seed extracts, the black currant seed oil also inhibited subcutaneous air pouch formed in Sprague-Dawley rats induced by monosodium urate crystals [Tate and Zurier, 1994]. The black currant seed oil enriched diet suppressed significantly both the cellular and fluid phases of inflammation (polymorphonuclear leukocyte and exudates accumulation). In contrast, administration of normal chow or of a diet enriched in sunflower oil containing the normal ratio of polyunsaturated fatty acid (PUFA) did not influence monosodium urate crystal-induced inflammation in this model [Tate and Zurier, 1994]. The findings indicate that a diet, which provides both n-6 (gammalinolenic acid) and n-3 (alpha-linolenic acid) fatty acids as substrates alternative to arachidonic acid for oxidative metabolism, can modify monosodium urate crystal-induced acute inflammation. Injection of both n-3 and n-6 polyunsaturated fatty acids induces higher anti-inflammatory responses than the effect obtained after treatment with either of them alone [Salem, 2000]. Similar to the black currant seed oil, *N. sativa* seeds contain both n-6 and n-3 fatty acids, it thus might also induce similar anti-inflammatory effects on monosodium urate crystal-induced acute inflammation.

Intensity of inflammatory immune responses is controlled by recruitment of inflammatory cells into inflammatory lesions. This process is tightly governed by expression of certain inflammatory chemokines, such as MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5).
[Kallinich et al., 2005 and Baggiolini and Dahinden, 1994] and adhesion molecules, such as LFA-1, CD62L and CD44, by the inflammatory cells, and ICAM-1 and VCAM-1 by the endothelial cells [Cartier et al., 2005]. Given the central role of chemokines and adhesion molecules in orchestrating the immune response, interference with the expression of these mediators substantially alter the quality of the immune response, leading to either enhancement or inhibition of the ongoing immune response. Thus, one potential mechanism that might mediate the inhibitory effect of N. sativa on inflammatory immune responses is an alteration of trafficking of the inflammatory cells via modulating expression of chemokines and/or adhesion molecules.

Immunomodulatory properties

Generation of effective immunity requires both innate immunity that recognizes pathogen associated molecular patterns and adaptive immunity that recognizes specific antigens [Medzhitov and Janeway, 2000]. Innate immunity consists of non-specific cells, including MΦ, granulocytes, NK cells and DCs. Adaptive immunity is comprised of a humoral arm mediated by B cells that secrete antigen-specific antibodies, and cellular arm mediated by CD4+ (helper) and CD8+ (cytolytic) T cells [Lucey et al., 1996]. CD4+ T helper cells are responsible for orchestrating an immune response, whereas cytolytic CD8+ T cells are the killer cells that traffic to sites of infection or cancer and lyse infected or tumor cells. Together, these two types of effector T lymphocytes play critical roles in eliminating infections and controlling cancer.

One of the precious properties of N. sativa is the immunomodulatory effects of its constituents. Studies begun just over a decade ago suggest that if it is used on an ongoing basis, N. sativa can enhance inhuman responses human. The majority of subjects who were treated with N. sativa oil for 4 weeks showed a 55% increase in CD4 to CD8 T cells ratio, and a 30% increase in natural killer (NK) cell function. The results have been presented by A. El-Kadi and O. Kandil to the 1st International Conference
on Scientific Miracles of Quran and Sunnah, held in Islamabad, Pakistan [Haq et. al., 1999]. Recently, a well-designed study analyzed the immunomodulatory effects of the whole extract of N.sativa seeds and their protein components in vitro [Haq et. al., 1999 Haq et. al., 1995]. By investigating the in vitro effects of the whole and soluble fractions of N.sativa seeds on human peripheral blood mononuclear cells (PBMC) response to different mitogens, the components did not show any significant stimulatory effect on the PBMC responses to the T cell mitogens phytohemagglutinin (PHA), or concanavalin-A (Con-A). By contrast, the components expressed stimulatory effect on the PBMC response to pooled allogeneic cells [Haq et. al, 1995]. Furthermore, in mixed lymphocyte cultures, four different purified proteins of N.sativa showed stimulatory effects. By contrast, a uniformly suppressive effect of the four fractions was noticed when lymphocytes were activated with the B cell mitogen PWM [Haq et. al., 1999]. Consistent with the stimulatory effects of N.sativa oil on proliferation of T cells, its ethyl-acetate column chromatographic fraction and water fraction enhance the proliferative response to ConA, but again not to the B cell mitogen LPS [Swamy and Tan, 2000]. These findings indicate that certain constitutions of N.sativa oil possess potent potentiating effects on the cellular (T cell-mediated) immunity, while other constituents possess suppressor effects on B cell-mediated (humoral) immunity. These findings suggest also that the stimulatory effects of N.sativa on the cellular immunity are dependent on the nature of the immune (e.g. ConA versus allogenic) response.

In line with the in vitro enhancing effects on N.sativa on the T cell immunity, in vitro studies confirm these effects. For instance, 1 week oral administration of aqueous extracts of N. sativum seeds increased [about 2-fold] the number of splenic NK cells, and their cytotoxicity against YAC-1 tumor targets when compared with control NK cells [Abuharfeil et. al, 2001]. In addition, oral administration of N.sativa oil commenced 6 weeks after induction of streptozotocin (STZ)-induced diabetes significantly induced beneficial effect, coincided with elevation in the phagocytic activity.
of peritoneal MΦ, and lymphocyte count in peripheral blood compared with untreated diabetic hamsters [Fararh et al., 2004], indicating to the potential of *N.sativa* oil to enhance functions of cells of innate-immunity, including MΦ and NK cells, as well as cellular immunity. Another example for enhancing immunity by *N.sativa* is its ability to ameliorate age-associated decline in T cell functions. Nutritional supplementation can enhance the immune response in elderly humans by changing both the total amount and the type of dietary lipids [Hummell, 1993]. *N.sativa* oil is rich in the n-6 PUFA α-linoleic acid (18:3n-6), the n-3 PUFA α-linoleic acid (18:3n-3) and a small amount of stearidonic acid (18:4n-3) [Laakso and Voutilainen 1996]. The composition of the seeds reflects the recommended optimal dietary intake of n-3 and n-6 fatty acids, i.e., it has a ratio of n-3 to n-6 fatty acids of 1 to 4 or 5 [Yehuda and Carasso, 1993]. Dietary supplementation with the *N.sativa* oil is found to improve the immune response of healthy elderly subjects, which is mediated by a change in the factors closely associated T cell activation [Wu et. al., 1999]. Delayed type hypersensitivity (DTH) skin tests have been widely used as an *in vivo* assay to determine cell-mediated immune function, and a decrease in DTH, is associated with increased morbidity and mortality [Christou et. al., 1989]. Treatment with *N.sativa* oil significantly increased the total diameter of indurations after 24 h of DTH induction in response to specific antigens (tetanus toxoid and T.mentagrophytes), when compared with presupplementation measurements or to the placebo group [Christou et. al., 1989].

In contrast to its enhancing effect on the T cell-mediated immune response, *N.sativa* constituents have shown a tendency to downregulate B cell-mediated immunity based on the results obtained from the *in vitro* experiments discussed earlier where *N.sativa* proteins suppressed PBMC responses to the B cell mitogens' LPS and PWM [Haq, 1995 Swamy and Tan, 2000]. One study confirmed this hypothesis *in vivo*, where the effect of the volatile oil of *N.sativa* seeds was studied on the antigen-specific response induced by vaccinating rats with the typhoid TH antigen. In that
study, treatment with *N. sativa* oil induced about 2-fold decrease in the antibody production in response to typhoid vaccination as compared to the control rats [Islam *et al.*, 2004]. Thus, based on the *in vitro* and *in vivo* data, it is likely that *N. sativa* constituent may enhance cellular immunity, while suppressing humoral immunity. Further studies, however, are required to validate this hypothesis, and to define the components responsible for each effect. Therefore, the immunomodulatory effects of this plant should be measured based on the nature of the immune response mediating the disease. Because PGE, LTB4, and mediators of oxidant stress down-regulate lymphocyte proliferation [Shapiro *et al.*, 1993 and Meydani *et al.*, 1990], and because *N. sativa* oil significantly decreases the production of these mediators.

Quality and quantity of cytokines are critical in initiation and execution of immunity. A variety of experiments have shown that excessive or insufficient production of cytokines may significantly contribute to the pathophysiology of a range of disease responses and are thought to be decisive for pathological or physiological consequences [Van der Meide and Schellekens, 1996]. After activation, CD4 T helper cells differentiate into either TH1-type cells, secreting IL-2, IL-12, IFN-γ and TNF-α, or TH2-type cells secreting IL-4, IL-5, IL-10, and IL-13. Indeed, the balance between TH1 and TH2 cytokines is critical for the orientation of the inflammatory response toward cell-mediated or humoral-mediated responses. Thus, any factor that can interfere with TH1/TH2 axis might affect the outcome of the response [Lucey *et al.*, 1996]. By investigating the effects of *N. sativa* seed proteins on cytokine production by humans PBMC, the proteins enhanced the production of IL-3 and IL-1 by lymphocytes when cultured with or without allogeneic cells [Haq *et al.*, 1995], suggesting the stimulatory effects of *N. sativa* seed proteins on the naive cell itself. However, under the same culture conditions, crude extract of *N. sativa* seeds or their soluble fractions did not show any effect on the production of IL-2 and IL-4 [Haq *et al.*, 1995]. Interestingly, even though *N. sativa* proteins suppressed the production of IL-8 in non-activated PBMC, they
did enhance its production by these cells when stimulated with PWM, a B cell mitogen. Of note, stimulatory effect of whole *N.sativa* and their fractionated proteins was also noticed on the production of TNF-α by either non-activated or mitogen-activated PBMC [Haq *et. al.*, 1999]. In a recent study, *N.sativa* oil exhibited a striking antiviral effect against murine cytomegalovirus infection coinciding with elevation of IFN-γ in serum, which lasted for a prolonged time [Salem and Hossain, 2000]. Thus, it is apparent that the effect of *N.sativa* on cytokine production depends on the nature and doses of ingredients and the nature of cytokines itself.

**Anti-microbial properties**

The findings discussed above indicate that *N.sativa* seed constituents possess potential immuno-modulatory effects, which as a consequence might impact on the host-parasite interrelationship. Consistent with this notion, the oil and active ingredients of *N.sativa* seeds have been reported to exert anti-microbial activities, including antibacterial, anti-fungal, anti-helminthic, and anti-viral effects [Salem and Hossain, 2000a Agarwal *et. al.*, 1979 Namba and Tsunezuka, 1985]. Some of these anti-microbial effects have been attributed to the immunomodulatory effects of *N.sativa* seed components. Ahmed John (2006 and 2007) reported that *eudrilus euginae* and *perionyx excavatus* show antimicrobial activity against pathogenic microbes.

**Anti-viral effect**

Murine cytomegalovirus (MCMV) is a herpes virus that causes disseminated and fatal disease in immunodeficient animals [Reynolds *et. al.*, 1993] similar to that caused by human cytomegalovirus in immunodeficient humans [Moro *et. al.*, 1999]. *In vivo* treatment with *N.sativa* oil induced a striking anti-viral effect against MCMV infection [Salem and Hossain, 2000a], indicating a promising therapeutic potential of *N.sativa* oil as an anti-viral remedy. Immunity generated toward viral infection is controlled by both the non-specific cells, including NK cells.
and Mφ, and specific cells including CD4 and CD8 T cells [Salem and Hossain, 2000]. Each cell population plays a central anti-viral role at a certain point of time post infection, where NK cells and Mφ are important during the early phase, while T cells are crucial for clearance of the virus at late stages [Su et. al, 2001]. Mediators produced by these cells mainly IFN-γ are seminal factors in mediation of the antiviral response. Interestingly, we found that the antiviral effect of the *N.sativa* oil is associated with enhancing response of CD4 and CD8 cells, and Mφ [Salem and Hossain, 2000], augmenting their ability of IFN-γ production that is known to render mice more resistance to MCMV infection [Yamaguchi et. al., 1988 and Orange et. al., 1995]. It has been reported that viral infection induces apoptosis leading to lymphocyte depletion in the host, and that anti-oxidant agents can inhibit virus-induced apoptosis as well as the viral replication in target cells [Peterhans, 1997]. Eventually, the anti-oxidant effect of the *N.sativa* oil may represent another mechanism that contributes to its anti-viral activity. Indeed, the anti-viral effects of *N.sativa* against MCMV infection open a new avenue for a novel anti-viral remedy.

**Anti-helminthic effects**

Schistosomiasis, a tropical parasitic disease, is endemic in the third world countries. Protection from this disease is mediated by both cellular and humoral immunity. Although vaccine trials have been tested, chemotherapy is still the only choice regimen to the human host [Chitsulo et. al., 2004]. *N.sativa* seed extracts and TQ have shown potential protective effects against *S. mansoni* infection [Aboul Ela, 2002]. Treatment of *S. mansoni*-infected mice with *N.sativa* oil induced reduction in the number of *S. mansoni* worms in the liver, coincided with a decrease in the egg burden in both the liver and the intestine. Importantly, the oil showed additive effects with praziquental, the drug of choice for the treatment of schistosomiasis [Mahmoud et. al., 2002]. Administration of *N.sativa* oil to *S. mansoni*-infected mice partially corrected the infection-caused alterations biochemical and pathological in ALT, GGT, and AP activities, as
well as the albumin content in serum [Mahmoud et. al., 2002 and Gharib et. al., 1999]. In murine schistosomiasis, a variety of cytokines are implicated as mediators of the granulomatous inflammatory response. Accordingly, modulation of cytokine levels can modify the intensity of the inflammatory response. Since *N. sativa* seeds increased the ratio of helper to cytotoxic T cells, and enhanced Mφ and NK cell activities in normal volunteers [Abuharfeil et. al., 2001] and in MCMV-infected mice [Salem et. al., 2000], and the production of IL-3 [Haq et. al., 1999 Haq et. al., 1995] and IFN-γ [Salem and Hossain, 2000], its anti-schistosome effect could in part be attributed to modulation of the immune response to schistosome eggs trapped in the liver. Similar to its anti-schistosome effects, the essential oil from the seeds of *N. sativa* showed pronounced anti-helminthic activity even in 1:100 dilution against tapeworms, earthworms, nematodes and cestode [Agarwal et. al., 1979].

**Anti-bacterial effects**

In addition to its anti-viral and anti-helminthic effects, *N. sativa* showed also anti-bacterial activity against several bacterial strains, including *Escherichia coli*, *Bacillus subtilis*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, as well as against the pathogenic yeast *Candida albicans* and fungus [Hanafy and Hatem, 1991 Khan et. al., 2003a and Morsi, 2000]. In an earlier study, DTQ showed anti-bacterial effect against the Gram-positive bacteria [El-Fatatry, 1975]; and diethyl ether extract caused concentration-dependent inhibition of the Gram-positive bacteria *Staphylococcus aureus*, and of Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Furthermore, the ether extract showed synergistic and additive antibacterial effect with several antibiotics [Hanafy and Hatem, 1991]. Importantly, the extract proved to be more effective against the drug resistant bacteria, including *V. cholera*, *E. coli* and all strains of *Shigella dysentriae* [Morsi, 2000]. Even though *in vitro* treatment of human PBMC with the soluble fractions of *N. sativa* seeds had no effect on the bacterial phagocytosis or killing activities of these cells when cultured with
*Staphylococcus aureus* [Haq *et al.*, 1995], *in vivo* treatment with the *N. sativa* seed diethyl ether extract successfully eradicated a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection [Hanafy and Hatem, 1991]. This might indicate that the bactericidal activity of *N. sativa* seed components observed *in vivo* is mediated by different host factors. Inoculum of *Candida albicans* into mice produces colonies of the organism in the liver, spleen, and kidneys. By studying anti-fungal effect of the aqueous extract of *N. sativa* seeds using this model, treatment of the infected mice daily for 3 days starting 24 h after inoculation of *C. albicans* markedly inhibited the growth of the fungus in all organs studied [Khan *et al.*, 2003a]. All the findings discussed above show that *N. sativa* seed constituents possess antimicrobial effects against different pathogens, including bacteria, viruses, helminths, and fungus. These findings are of a great practical significance, since *N. sativa* seeds have been traditionally and clinically used in Middle and Far Eastern countries without any reported undesirable effects. It may thus be valuable as a co-therapeutic agent against different microbes.

**Anti-tumor properties**

*In vitro* anti-tumor effects

*In vitro* and *in vivo* studies indicate that both the oil and the active ingredients of *N. sativa* seeds possess anti-tumor effects. By investigating the effect of the volatile oil of *N. sativa* seeds on different human cancer cell lines, the oil expressed marked cytotoxic effects against a panel of human cancer cell lines [Islam *et al.*, 2004]. Exposure of MCF-7 breast cancer cells to aqueous and alcohol extracts alone or in the presence of descending potency for H$_2$O$_2$ completely inactivated growth of these cells [Swamy and Tan, 2000], suggesting that *N. sativa* alone or in combination with oxidative stress is an effective anti-cancer agent. Studies attempted to define the anti-tumor mechanisms of the whole *N. sativa* oil show that *N. sativa* extracts induced, in a concentration-dependent manner, inhibition of the metastasis-induced factors, including type 4 collagenase, metalloproteinase, and serineproteinase inhibitors [Medenica *et al.*, 1997],

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angiogenic protein-fibroblastic growth factor [Swamy and Tan, 2003], tissue-type plasminogen activator, urokinase-type plasminogen activator, and plasminogen activator inhibitor type 1 [Awad, 2005]. Because tumor cells to ensue their metastasis produce these factors, it can be suggested that the anti-tumor effects of N.sativa oil might be mediated through anti-angiogenic effects through inhibition of local tumor invasion and metastasis in vivo.

In addition to the anti-tumor effects of the whole extract of N.sativa, TQ, DTQ, and other active ingredients also showed cytotoxic effects. For instance, the active ingredient extracted by ethyl-acetate column chromatographic fraction 5 (CC-5), or α-hedrin, expressed anti tumor effects against different cancer cell lines with selectivity against hepatocellular carcinoma, leukemic cell, Lewis lung carcinoma [Swamy and Tan 2000], and leukemia cells through a rapid depletion of intracellular GSH and disruption of mitochondrial membrane potential with subsequent increase in the production of reactive oxygen species [Swamy and Huat, 2003]. Both TQ and DTQ were equally cytotoxic against different human tumor cells lines, including the pancreatic adenocarcinoma, human uterine sarcoma and human leukemic [Salomi et al., 1992 and Worthen et al., 1998], triggering their apoptosis through arresting the growth of these cells in G1 phase of the cell cycle [Gali-Muhtasib et al., 2004] associated with increase in the gene and protein expression of p53 and inhibition of the anti-apoptotic Bcl-2 protein. This indicates that the anti-neoplastic effect of TQ is mediated by pro-apoptotic effects modulated by Bcl-2 protein and is linked to and dependent on p53.

In vivo anti-tumor effects

The reported in vitro anti-tumor effects of the N.sativa oil and its active ingredients have also been confirmed in vivo in different tumor models. For instance, topical application of N.sativa inhibited two-stage initiation/promotion anthracene/croton oil skin carcinogenesis induced in mice by 7,12-dimethylbenz(a)anthracene//croton oil in mice, where the
onset of papilloma formation was delayed, and the mean number of papillomas was reduced. The active principle fatty acids derived from *N. sativa*, completely inhibited the growth of Ehrlich ascites carcinoma and Dalton's lymphoma ascites cells [Salomi et al., 1992]. Moreover, oral feeding with *N. sativa* extract suppressed hepatic tumor in rat induced by diethylnitrosamine or by partial hepatectomy [Iddamaldeniya et al., 2003]. Furthermore, *N. sativa* oil suppressed colon carcinogenesis induced by methyl nitrosourea [Mabrouk et al., 2002] or by 1,2-dimethylhydrazine [Salim and Fukushima, 2003]. In the latter study, administration of *N. sativa* oil given during the post-initiation stage markedly decreased the total number of aberrant crypt foci through anti-proliferative activity. In addition, α-hederin, another ingredient of the crude extract of *N. sativa* oil, was also found to show *in vivo* anti-tumor activity against leukemia and Lewis lung carcinoma [Kumara and Huat, 2001], prolonging the life span of the tumor-bearing mice.

The anti-tumor effects of *N. sativa* oil might be attributed to the effect of TQ, since administration of TQ in drinking water resulted in significant suppression of forestomach tumor induced by benz(a)pyrene [Badary et al., 1999]. Similarly, the same treatment regimens of TQ significantly inhibited the tumor incidence and tumor burden of 2-methylnitrosourea induced soft tissue fibrosarcoma [Badary and Gamal El-Din, 2001] associated with reduction in hepatic lipid peroxides and increased enzyme contents and activities of GST and GSH. Using the same fibrosarcoma tumor model, administration of *N. sativa* extract 30 days after subcutaneous administration of methylnitrosourea restricted fibrosarcoma tumor incidence to 33.3%, compared with 100% in control tumor-bearing mice [Salomi et al., 1991], indicating therapeutic potentials. Furthermore, oral administration of TQ to mice bearing Ehrlich ascites carcinoma xenograft significantly enhanced the anti-tumor effect of ifosfamide, coincided with less B.Wt.loss and mortality rate [Badary, 1999 Badary et al., 1999 and Badary and Gamal El-Din, 2001]. Interestingly, TQ protects against doxorubicin-induced cardiotoxicity without compromising its anti-
tumor activity [Al-Shabana et al., 1998]. These observations demonstrate that TQ, in addition to its prophylactic and therapeutic anti-tumor effects, can be a potential chemotherapeutic adjuvant to standard chemotherapy. This might lower the does of standard chemotherapeutic drugs, while augmenting their anti-tumor efficacy.

As discussed earlier, it became known that suppression of immune cell function associated with chemotherapy [Billau et al., 2003 Angulo et al., 2000], radiotherapy [Billiau et al., 2003], and late stages in tumor-bearing hosts [Kusmartsev et al., 2000] is mediated, at least in part, by NO produced by immature Ly6G+CD11b+ granulocytes that are massively generated under these conditions [Angulo et al., 2000 Mazzoni et al., 2002 and Dupuis et al., 2003]. Therefore, it is possible that the anti-tumor effects reported for N.sativa oil and TQ are mediated by their abilities to scavenging the NO produced by these cells. The impact of N.sativa ingredient, in particular TQ, on these cells in the tumor-bearing hosts needs to be explored. In addition, since chemotherapy induces massive expansion of the immature granulocytes, which produce large amount of NO, it might be feasible to follow chemotherapy with TQ treatment that might alleviate the suppressive effects on the immune responses by chemotherapy-induced NO. In addition to the possible anti-oxidant mediating anti-tumor effects of TQ, it is also possible that its anti-tumor effects if mediated by the ability to suppress PEG and LT. Higher levels of these inflammatory mediators have been reported to correlate with tumor progression in vivo [Gately and Kerbel, 2003], and several drugs that are able to block the eicosanoid signalling, both COX-1 and COX-2 pathways, are being tested now in clinical trials [Gately and Kerbel, 2003 Kundu and Fulton, 2002]. However, the possibility that both the anti-oxidant and anti-inflammatory effects of TQ mediate its anti-tumor effects needs to be directly tested by using mice that are knocked out for these mediators.

Taken together, the findings of these studies indicate the potential of the active ingredients of N.sativa oil, in particular TQ, as a powerful
chemopreventive agents against several experimental cancer, including fore-stomach, fibrosarcoma, colon, skin, and hepatic tumors.

**Anti-analgesic properties**

In Saudi Arabia and neighboring countries *Nigella sativa* oil is used as a topical treatment for pain and stiffness in joints. This indication together with its use in bronchial asthma suggested for the investigation of analgesic and anti-inflammatory effects of *Nigella sativa*. Houghton et al (1995) reported that crude fixed oil of *Nigella sativa* and an active principle, thymoquinone, inhibited cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism in rat peritoneal leukocytes. The effect was demonstrated via the dose-dependent inhibition of the formation of thromboxane B2 and leukotriene B4. This effect was later confirmed in experimental animal studies conducted by Mutabagani and El-Mahdy (1997) using *Nigella sativa* volatile oil A1-Ghamdi (2001) using aqueous suspension of *Nigella sativa* crushed seeds. In both studies formation of edema in rat hind paw was inhibited and these effects were comparable to those of indomethacin and aspirin, respectively. A1-Ghamdi, (2001) also reported an analgesic effect of aqueous suspension of *Nigella sativa* seeds, comparable to aspirin, as measured by hot plate test conducted in rats. However, the suspension did not relieve yeast-induced pyrexia in rats. The mechanism of anti-inflammatory and analgesic effects seems to be related to the inhibition of eicosanoid synthesis as suggested by the study of Houghton et al (1995). Another possibility for the analgesic action could be the activation of supraspinal μ (1)- and κ-opioid receptors subtypes, as elicited by the antagonistic effect of naloxone, naloxonazine and nor-binaltorphimine to antinociceptive effects of *Nigella sativa* oil and thymoquinone (Abdel – Fattah et. al., 2000).

**Effects on the Respiratory System**

In Saudi Arabia and neighboring countries *Nigella sativa* seeds and oil are commonly used for the treatment of asthma. Nigellone (a carbonyl polymer of thymoquinone) proved to be an excellent prophylactic agent for
both bronchial asthma and asthmatic bronchitis and was more effective in children than adults (Badar El-Din, 1960 and Mahfouz and El-Dakhakhany, 1990). El-Sayed et al., (1994) has also reported the use of *Nigella sativa* in asthma in the traditional medicine. However, El-Tahir et al., (1993a) observed that *Nigella sativa* volatile oil induced dose dependent increase in the respiratory rate and the intra-tracheal pressure, which were antagonized by mepyramine, atropine and reserpine but not by indomethacin, diethyl-carbamazine or hydrocortisone. A central mechanism was suggested for these effects. Apparently, these observations seem to be in contrary to the antihistaminic effect reported by El-Dakhakhany (1982) and its use in the folk medicine for asthma. However, in a later study, aqueous extract of *Nigella sativa* competitively, and the macerated extract non-competitively antagonized methacholine induced contractions of isolated guinea-pig tracheal chain (Boskabady and Shahabi, 1997). Similarly, crude extract of *Nigella sativa* has also been shown to cause relaxation of carbachol, histamine, and K+ induced contractions of isolated guinea-pig trachea (Gilani et al., 2001).

**Effect on Cardiovascular System**

In Arabian folk medicine whole seeds of *Nigella sativa* alone or in combination with honey or garlic are promoted for the treatment of hypertension, which drew the attention of EL-Tahir et al., (1993a) to investigate the effects of *Nigella sativa* on the cardiovascular system. Volatile oil and thymoquinone produced a dose dependent decrease in the arterial blood pressure and the heart rate. These effects were significantly antagonized by atropine, cyproheptadine, hexamethonium and spinal pithing. However, reserpine only antagonized the effects of low doses of volatile oil but not of thymoquinone. They suggested that these effects were centrally mediated, mainly via the involvement of both the 5-hydroxytryptaminergic and muscarinic mechanisms. Similarly, an oral dose of 0.6ml/kg/day of *Nigella sativa* extract produced a significant hypotensive effect in spontaneously hypertensive rats comparable to that of 0.5mg/kg/day of oral nifedipine (Zaoui et al., 2000 and 2002). This
effect was concluded to be partially due to the diuretic effect of *Nigella sativa*, which was comparable to 0.5 mg/kg/day furosemide or by other mechanisms as mentioned above.

**Effect on Genito-Urinary System**

In Unani medicine *N. sativa* is promoted for the treatment of oligomenorrhea, to induce menstruation and to treat infertility (Al-Jishi, 2000). El-Mahmoudy *et al.*, (2002) reported that *Nigella sativa* crude oil induced uterine contractions both *in vivo* in pregnant rabbits and *in vitro* of non-pregnant rat uteri. Similarly, Keshri *et al.*, (1995) found that the hexane extract of *Nigella sativa* exhibited mild uterotropic activity and prevented pregnancy in rats when given on day 1-10 post-coitum. On the contrary, Aqel and Shaheen (1996) reported that volatile oil of *Nigella sativa* inhibited spontaneous contractions of rat and guinea pig uterine smooth muscle and those induced by oxytocin. Similarly, treatment of pregnant rats with fixed oil of *Nigella sativa* for 2 weeks significantly suppressed PGE2 and oxytocin – induced contractions of isolated rat uteri treated with diethylstilboesterol, suggesting the potential use of *Nigella sativa* oil in the uterine disturbances associated with prostaglandin and oxytocin induced increased contractility e.g. some dysmenorrhoeas, premature deliveries and habitual abortions (El – Tahir *et al.*, 1999). These differences may be due to the different doses, preparations and the animal species used.

**Effect on Gastro-Intestinal Tract**

In Unani medicine *Nigella sativa* is used for stomachache and as a digestive, carminative, laxative and anti-jaundice (El – Kadi and Kandil, 1986). Oral *Nigella sativa* powder was reported to relieve flatulence by Chopra *et al.*, (1956). While Nigellone, an active principle of *N. sativa* was found to antagonize histamine induced contractions of guinea pig intestine. In addition, El-Dakhakhani (1965) reported a choleretic effect of *Nigella sativa* oil and its active principles (thymoquinone, thymohydroquinone and dithymoquinone), respectively. El-Dakhakhani
et. al., (2000) investigated the effect of *Nigella sativa* oil on gastric secretion and ethanol - induced ulcer in rats. Significant increase in mucin content, glutathione level as well as a significant decrease in mucosal histamine content and ulcer formation, with a protection ration of 53.56%, was found in the *Nigella sativa* oil pretreated group. More recently, the crude extract of *Nigella sativa* was shown to cause a dose – dependent (0.1 – 3.0mg/ml) relaxation of spontaneous contractions of rabbit jejunum as well as inhibition of K+ - induced contractions in a similar dose range, suggestive of calcium channel blockade (Gilani et. al., 2001).

**Hypoglycemic effect**

Al-Awadi and Gumma, (1987) have reported the use of a plant mixture containing *Nigella sativa*, Myrr, Gum Olybanum, Gum Asafoetida and Aloe by diabetics in Kuwait. They confirmed the blood glucose lowering effect of *Nigella sativa*, in combination with other herbs in rats. The mechanism of action was later investigated and appeared to be due to the inhibition of hepatic gluconeogenesis (Al – Awadi et. al., 1991). The volatile oil of *Nigella sativa* alone also produced a significant hypoglycemic effect on normal and alloxan- induced diabetic rabbits without changes in insulin levels (Al – Hater et. al., 1993). The hypoglycemic effect of *Nigella sativa* in combination with other herbs on alloxan-induced diabetic rats was also reported by Eskander et al (1995) and El-Shabrawy and Nada (1996). Furthermore, Bamosa et al (1997) reported a significant decrease in blood sugar of healthy human volunteers treated with 1gram of *Nigella sativa* capsules twice daily.

**Effect on Blood**

In Kuwait some people use extract of *Nigella sativa* with natural fat for epistaxis. In view of that the petroleum ether extract of *Nigella sativa* was studied for its action on blood coagulation and was reported to shorten the whole blood clotting time, plasma clot time and kaoline – cephalin clotting time of male rabbits when compared to control. In addition, a significant shortening of bleeding time in rats was also
observed. However, there were no significant effects on the thrombin time or prothrombin time but the partial thromboplastin time was shortened while euglobulin time was prolonged (Ghoneim et al., 1982). In contrast, *Nigella sativa* fixed oil suppressed adenosine diphosphate – induced platelet aggregation in both normal and diabetic rats (El - Tahir et al., 1999). Similarly, in a recent study it was observed that the menthol soluble components of *Nigella sativa* oil including 2 - (2 - Methoxypropyl) – 5 Methyl – 1, 4 – benzene diol, thymol and carvacrol as well as eight other related compounds had very strong inhibitory effect on arachidonic acid induced platelet aggregation. This platelet aggregation inhibitory effect was more potent than that of aspirin (Sayed, 1980). Bamoso et al., (1997) reported a pattern of decrease levels of cholesterol and triglycerides (on days 7 and 14) of healthy human volunteers treated with 1 gram of *Nigella sativa* capsules twice daily. This effect was later confirmed by (E1 – Dakkhani et al., 2000) using *Nigella sativa* oil (800mg/kg orally for 4 weeks) in rats showing a significant decrease in serum total cholesterol, low density lipoprotein and triglycerides and an elevation of serum high density lipoprotein level. More recently, *Nigella sativa* oil in rats has been shown to decrease the serum cholesterol, triglycerides and glucose levels as well as the counts of leukocytes and platelets by 15.5,22,16.5,35 and 32% respectively while haematocrit and haemoglobin levels increased by 6.4 and 17.45 respectively (Zaoui et al., 2002). However, A1 – Jishi, (2000) did not find any change in blood cells when *Nigella sativa* was given to normal rats.

**Potential toxicity of *N.sativa* seeds**

All the above information revealed the beneficial immunotherapeutic potentials of the crude oil and extracts of *N.sativa* seeds and its active ingredient TQ toward several disease settings. However, toxicity of medicinal plants is central for acceptance of their therapeutic application in human. In one study, potential toxicity of the fixed oil of *N.sativa* seeds was investigated in mice and rats through determination of LD50 values and examination of possible biochemical, hematological and
Selected studies showing the different doses and routes of administration of *N. sativa* seed grains and extracts tested in experimental models *in vivo*

<table>
<thead>
<tr>
<th>Dose</th>
<th>Route</th>
<th>Model</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20, 200 g/kg</td>
<td>Diet</td>
<td>Toxicity</td>
<td>Chicks</td>
<td>[Al-Homidan et al., 2002]</td>
</tr>
<tr>
<td>0.2 g/day</td>
<td>Oral</td>
<td>Methylnitrosurea induced colon cancer</td>
<td>Rats</td>
<td>[Mabrouk et al., 2002]</td>
</tr>
<tr>
<td>Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6 ml/kg</td>
<td>Oral</td>
<td>Candidiasis infection</td>
<td>Mice</td>
<td>[Khan et al., 2003]</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>Oral</td>
<td>KBrO3 induced toxicity</td>
<td>Rats</td>
<td>[Khan et al., 2003]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Topical</td>
<td>Skin carcinogenesis</td>
<td>Mice</td>
<td>[Salomi et al., 1991]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Oral</td>
<td>Ehrlich ascites carcinoma</td>
<td>Mice</td>
<td>[Salomi et al., 1992]</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>Oral</td>
<td>Carrageenan induced oedema</td>
<td>Mice</td>
<td>[Al-Ghamdi, 2001]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>i.p.</td>
<td>Nociceptive activities</td>
<td>Mice</td>
<td>[Al-Naggar et al., 2003]</td>
</tr>
</tbody>
</table>
Selected studies showing the different doses and routes of administration of *N. sativa* seed oil tested in experimental models *in vivo*

<table>
<thead>
<tr>
<th>Dose</th>
<th>Route</th>
<th>Model</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mg/kg</td>
<td>i.p.</td>
<td>MCMV (virus) infection</td>
<td>Mice</td>
<td>[Salem and Hossain, 2000]</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>Oral</td>
<td>Colon Carcinoma</td>
<td>Rats</td>
<td>[Salim and Fukushima, 2003]</td>
</tr>
<tr>
<td>0.5-2 ml/kg</td>
<td>Oral</td>
<td>Gentamicin induced toxicity</td>
<td>Rats</td>
<td>[Ali, 2004]</td>
</tr>
<tr>
<td>2.5, 5 mg/kg</td>
<td>Oral</td>
<td>Schistosoma mansoni infection</td>
<td>Mouse</td>
<td>[Mahmoud et al., 2002]</td>
</tr>
<tr>
<td>180 mg/kg</td>
<td>Diet</td>
<td>Homeostasis</td>
<td>Rats</td>
<td>[Al-Jishi and Abu Hozafa, 2003]</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>i.p.</td>
<td>Cisplatin induced toxicity</td>
<td>Rats</td>
<td>[El Daly, 1998]</td>
</tr>
<tr>
<td>4-32 Al/kg</td>
<td>i.v.</td>
<td>Urethane anaesthetization induced respiratory pressure</td>
<td>Guinea pigs</td>
<td>[El Tahir et al., 1993]</td>
</tr>
<tr>
<td>2.5, 5 ml/kg</td>
<td>Oral</td>
<td>Ischemia/reperfusion induced gastric lesion</td>
<td>Rats</td>
<td>[El-Abhar et al., 2003]</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>Oral</td>
<td>CCl4 induced toxicity</td>
<td>Rats</td>
<td>[El-Abhar et al., 2003]</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>i.p.</td>
<td>STZ induced diabetes</td>
<td>Mice</td>
<td>[Fararh et al., 2004]</td>
</tr>
<tr>
<td>100,400 µl/kg</td>
<td>Oral</td>
<td>Carrageenan induced oedema/croton oil-induced ear oedema</td>
<td>Rats</td>
<td>[Hajhashemi et al., 2004]</td>
</tr>
<tr>
<td>0.2 ml/kg</td>
<td>Oral</td>
<td>Thymoid immunization/Abs</td>
<td>Mice</td>
<td>[Islam et al., 2004]</td>
</tr>
<tr>
<td>0.2 ml/kg</td>
<td>i.p.</td>
<td>STZ induced diabetes</td>
<td>Rats</td>
<td>[Kanter et al., 2004]</td>
</tr>
<tr>
<td>0.2 ml/kg</td>
<td>i.p.</td>
<td>CCl4 induced toxicity</td>
<td>Rats</td>
<td>[Kanter et al., 2003]</td>
</tr>
<tr>
<td>2 g/kg</td>
<td>Oral</td>
<td>Ant fertility against pregnancy</td>
<td>Rats</td>
<td>[Keshri et al., 1995]</td>
</tr>
<tr>
<td>50,400 mg/kg</td>
<td>Oral</td>
<td>Nociceptive induced insults</td>
<td>Mice</td>
<td>[Abdel-Fattah et al., 2000]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Oral</td>
<td>Methionine induced HHcy</td>
<td>Rats</td>
<td>[El-Saleh et al., 2004]</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>Oral</td>
<td>Blood homeostasis</td>
<td>Rats</td>
<td>[Zaoui et al., 2002]</td>
</tr>
</tbody>
</table>
Selected studies showing the different doses and routes of administration of TQ, the active ingredients of *N. sativa* seeds, tested in experimental models *in vivo*.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Route</th>
<th>Model</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5–10 mg/kg</td>
<td>Oral</td>
<td>Nociceptive-induced insults</td>
<td>Mice</td>
<td>[Abdel-Fattah et al., 2000]</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>Oral</td>
<td>Ifosfamide-induced FS</td>
<td>Rats</td>
<td>[Badary, 1999]</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Oral</td>
<td>Ehrlich ascites carcinoma</td>
<td>Mice</td>
<td>[Badary et al., 1999]</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Oral</td>
<td>DOX-induced toxicity</td>
<td>Rats</td>
<td>[Badary et al., 2000]</td>
</tr>
<tr>
<td>0.01%</td>
<td>Oral</td>
<td>Benzo(a)pyrene-induced stomach tumor</td>
<td>Mice</td>
<td>[Badary et al., 1999]</td>
</tr>
<tr>
<td>0.01%</td>
<td>Oral</td>
<td>Methylocholanthrene induced sarcoma</td>
<td>Mice</td>
<td>[Badary and El-Din, 2001]</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>i.v.</td>
<td>Arterial blood pressure</td>
<td>Rats</td>
<td>[163]</td>
</tr>
<tr>
<td>1.6–6.4 mg/kg</td>
<td>Oral</td>
<td>Urethane anaesthetization induced respiratory pressure</td>
<td>Guinea pigs</td>
<td>[El-Tahir et al., 1993]</td>
</tr>
<tr>
<td>5–100 mg/kg kg</td>
<td>Oral</td>
<td>Ischaemia/reperfusion induced gastric lesion</td>
<td>Rats</td>
<td>[El-Mahmoudy et al., 2002]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Oral</td>
<td>Methionine induced HHcy</td>
<td>Rats</td>
<td>[El-Saleh et al., 2004]</td>
</tr>
<tr>
<td>5–10 mg/kg</td>
<td>Oral</td>
<td>Acetic acid-induced colitis</td>
<td>Rats</td>
<td>[Mahgoub, 2003]</td>
</tr>
<tr>
<td>4–50 mg/kg</td>
<td>i.p.</td>
<td>CCl4 induced toxicity</td>
<td>Mice</td>
<td>[Mansour et al., 2001]</td>
</tr>
<tr>
<td>78–103 mg/kg</td>
<td>i.p.</td>
<td>Determination of LD50=90 mg/kg</td>
<td>Mice</td>
<td>[Mansour and Tornhamre, 2004]</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>i.v.</td>
<td>Inflammation (EAE model)</td>
<td>Mice</td>
<td>[Nieto et al., 2003]</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Oral</td>
<td>CCl4 induced toxicity</td>
<td>Rats</td>
<td>[Nagi et al., 1999]</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Oral</td>
<td>DOX induced toxicity</td>
<td>Rats</td>
<td>[Nagi and Mansour, 2000]</td>
</tr>
</tbody>
</table>
histopathological changes [Zaoui et al., 2002]. LD50 values, obtained by single doses (acute toxicity) in mice, were 28.8 ml/kg body with oral administration, and 2.06 ml/kg body with intraperitoneal administration. Chronic toxicity was studied in rats treated daily with an oral dose of 2 ml/kg body wt. for 12 weeks. Changes in key hepatic enzyme levels, including ALT, AST, and GSH, and histopathological modifications (heart, liver, kidneys and pancreas) were not observed in rats treated with \textit{N.sativa} oil after 12 weeks of treatment. Of note, however, the serum cholesterol, triglyceride and glucose levels and the count of leukocytes and platelets decreased significantly, compared to control values, while hematocrit and hemoglobin levels increased significantly. A slowing of B.Wt.gain was also observed in \textit{N.sativa}-treated rats compared to control animals. Consistent with this non-toxic effect of \textit{N.sativa}, it has been reported recently that treatment of Fischer 344 rats with the crude oil of \textit{N.sativa} for 14 weeks did not induce pathological changes in the liver, kidneys, spleen, or other organs [Salim and Fukushima, 2003] nor the biochemical parameters of blood and urine as well as B.Wt.gain. Further analysis on the potential toxicity of \textit{N.sativa} seeds revealed that feeding Hibro broiler chicks diet containing 20 or 100 g/kg \textit{N.sativa} seed ground for 7 weeks did not adversely affect growth.

Taken together, the parameters emerged from these studies indicate that \textit{N.sativa} is not toxic, as evidenced by high LD50 values, hepatic enzyme stability and organ integrity, suggesting a wide margin of safety for the therapeutic doses of \textit{N.sativa} fixed oil. However, the changes in hemoglobin metabolism and the fall in leukocyte and platelet count must be taken into consideration. In addition, the route of administration of \textit{N.sativa} seems to be crucial for its toxicity, since the LD50 was higher with oral administration (a 20-fold higher) than with intraperitoneal route [Zaoui et al., 2002], indicating that oral intake is safer than the systemic one.