Introduction
CHAPTER 1

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Liver and life

Liver is an exquisitely simple and extraordinarily complex organ; simple because the parenchyma is composed of a single epithelial cell type, the hepatocyte, arranged as sheets intimately associated with a venous portal system in an uncomplicated and consistent way throughout the organ; complex because these hepatocytes perform over the five hundred metabolic processes that include functions essential for life, others that are important for maintaining homeostasis and those that contribute to general good health (Cardell and Cardell, 1997).

Broad categories of important hepatocyte functions include carbohydrate metabolism; synthesis and secretion of proteins; establishment; maintenance and regulation of a large metabolic pool related to the carbohydrate, protein and lipid building blocks utilized throughout the body; storage of glycogen, fat and vitamins; inactivation of steroid and polypeptide hormones; processing of certain endogenous wastes and detoxification of metabolic and exogenous "Poisons". Owing to liver's position between the gut and the heart in the portal circulatory system and hepatocyte intimacy with thin-walled sinusoids, liver serves as a filter of blood, removing nutrients, anabolic and catabolic products and toxins, a regulator of blood plasma volume and blood levels of glucose, cholesterol and ammonia and a significant mediator of electrolyte and water balance (Netter, 1979).

Liver, the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Thus disorders associated with this organ are numerous and varied. Injury of hepatocytes, which may be reversible or irreversible is not only an important phenomenon in primary liver diseases and also may be significant as a secondary factor in other human diseases. Injury of sufficient severity and duration results in
irreparable or irreversible disruption of cell integrity leading to cell death and necrosis.

**Hepatic Injury**

Hepatic toxicity inflicted by foreign, natural chemicals and pathogens has been recognized for slightly over a century. The susceptibility of liver to injury by such agents appears to be a consequence of an anatomical position of this organ and the central role it plays in the metabolism and disposition of foreign chemicals. A number of features predispose the liver to injury, because the predominant blood supply of liver first passes through the intestines it is low in oxygen but highly enriched in nutrients. Likewise it also contains endotoxin (Lipopolysaccharide products of intestinal bacteria), metabolic waste products, adsorbed chemicals, entered microbes and other cell debris which may all enter the liver via the portal circulation and present a risk of toxicity to the liver as well as other organs. Following oral ingestion, toxicants can achieve high concentration in the liver (Koporec et al., 1995).

**D Galactosamine and lipopolysaccharide**

D-galactosamine is an amino sugar normally found *in vivo* only in acetylated form in certain structural polysaccharides. Administration of single dose of this compound to certain species results in dose-dependent hepatic damage resembling viral hepatitis, with focal necrosis and periportal inflammation (Timbrell and Franscis, 1991). It induces hepatotoxicity by inhibiting the synthesis of RNA and protein through a decrease in cellular UTP concentration (Zbigniew Kmiec et al., 2000), which finally leads to the necrosis of liver cells (Decker and Keppler, 1974).

Lipopolysaccharide (LPS) is a component of gram-negative bacteria that elicits a potent inflammatory response in mammals. In larger doses LPS causes liver injury that is dependent on a multitude of inflammatory factors including kupffer cells, neutrophils, platelets, cytokine, and
components of coagulation cascade particularly thrombin. At smaller
doses, LPS increases liver sensitivity to Galactosamine, ethanol, carbon
tetra chloride, aflotoxin B₁, monocrotaline and allyl alcohol (Barton et al.,
2001). Fulminant hepatitis can be induced in experimental animals by the
synergistic action of a small dose of lipopolysaccharide and D-
Galactosamine (an inhibitor of hepatocellular RNA synthesis).

Hepatoprotection

The liver damage caused by pathogens as well as chemical agents is
of similar nature and a proper treatment regime or plan is absent for both.
The lack of reliable liver protective drugs in allopathic medicine is explicitly
inadequate. The modern medicine offers only prophylactic immunization
against hepatic toxicity, but curative medicines with therapeutic rationale
are not available till now. Inspite of spectacular advances in modern
medicine, numerous problems still remain, especially the management of
liver disorder is still empirical. The drugs available in the modern
medicinal system for providing protection against these disorders are
inadequate and at best provide only symptomatic relief. However in the
traditional systems a number of drugs of natural origin are claimed to
provide protection against the hepatic disorders.

Spices

Spices are defined by the US Food and Drug Administration (2002)
as "aromatic vegetable substances, in the whole, broken, or ground forms,
whose significant function in food is seasoning rather than nutrition. They
are true to name, and from them no portion of any volatile oil or other
flavoring principle has been removed".

Spices are extracted from different plant parts such as rhizomes
(ginger and turmeric), leaves (mints and tejpat), fruits (cardamom and
chilies), berries (pepper), seeds (Black seed and coriander), kernel
(nutmeg), aril (mace), bark (cinnamon), bulbs (onion and garlic) and floral
parts (clove and saffron). Generally, spices are classified according to
The importance of spices is:

To serve as appetizers; therefore they are also termed as 'food adjuncts' or 'accessories'.
To add a tang and flavour to insipid or bland food.
To impart flavour to beverages.
To possess antioxidant properties.
To possess strong antimicrobial and antibiotic activities.
To have medicinal value.
To act as mouth freshener, clean the oral cavity from adhesion and bacteria.
As ingredient of some cosmetics and
To be used as preservatives in pickles and chutneys.

Given the wide range of botanical species and plant parts from which spices are derived, spices can contribute the significant variety and complexity to the human diet. In the past, the medicinal uses of spices and herbs were often indistinguishable from their culinary uses; people have recognized for centuries both the inherent value, as well as potential toxicity, of phytochemicals in relation to human health. Today, in the areas of AIDS and cancer prevention, the use of spices and their constituents as potential chemopreventive agents remains a topic of intense research.

**Nigella sativa**

*Nigella sativa* is an annual herbaceous plant belonging to the Ranunculaceae family, growing in Mediterranean countries, in the near east and in India. It is slightly bitter and peppery with a crunchy texture. Seeds are angular, of generally small size (1-5 mg), dark gray or black in colour and corrugated (Al-Gaby, 1998). Flowers are bluish, with a variable
number of sepals and characterized by the presence of nectaries. *Nigella Sativa* seeds are used for edible and medicinal purposes in many countries. They are used as a condiment in bread and other dishes. They are also used in the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey and in flavouring foods.

**Constituents**

*Nigella sativa* contains over 100 valuable nutrients. The contents are similar to evening primrose oil, because of its complex composition, it is much stronger. The active ingredient of *Nigella sativa* is the thymoquinone. It also contains in significant proportions protein, carbohydrate and essential fatty acids (Mohamed Bassim Atta, 2003). Other ingredients include linoleic acid, oleic acid, calcium potassium, iron, zinc, magnesium, selenium, vitamin A and vitamin E.

**Nigella sativa And the Present Study**

In the present investigation *Nigella sativa* L. seeds (Black Cumin) has been selected. The obvious reason in choosing this spice is multipurpose.

- It is natural and therefore a healthier approach to enhance the detoxifying efficiency by protecting the liver without side effects (Al-Gharably et al., 1997 and Nagi et al., 1999).

- It is well known for its flavour world over and is accepted by common man as a spice. (Mahfouz and El-Dakhakhany, 1990).

- It has been reported to possess a number of medicinal properties: The antioxidant (El-Tahir et al., 1993) antitumor (Worthen et al., 1998), antibacterial (Ferdous et al., 1992), anti-inflammatory (Houghton et al., 1995), hypocholesterolemic (Bamosa et al., 1997), anti allergic (Gore and custoric, 2004), hypotensive (Zaoui et al., 2000), immunomodulatory (Chakravarty, 1993; Haq et al., 1999; Swamy and Tan, 2000) and hepatoprotective (Daba and Abdel-Rahman, 1998). The screening of *Nigella sativa* for the hepatic toxicity is mandatory. Hepatotoxicity of some of the
herbal products has also been documented for many years. In western
countries, cases of herb-induced hepatitis have been observed after use of
preparations containing Symphytum or Chinese herbs. *Atractylis
gummiferai* and *Atractylis germander* are other examples of herbs inducing
hepatotoxicity. Cases of fatal liver accidents have been reported after
ingesting *Callilepis laureola*, a herb used by the Zoulous in Natal for
medicinal purposes or after use of products containing extracts of
*Teucrium chamaedrys*, which was nevertheless authorized in France in
1986 for use in preparations for weight loss (Larrey, 1994).

**Aim and Objectives**

The study of natural products of potential therapeutic values has
been and is of great fascination not only because of the medicinal
application of the active principles involved, but also equally as an
intellectual exercise in understanding nature's process and purpose. With
the high prevalence of viral B hepatitis in India, screening the
hepatoprotective activity of a drug by the mould of D-
galactosamine/Lipopolysaccharide induced liver damage is meaningful
since the liver injury mode closely mimics all features of viral hepatitis and
the study will rationalize the traditional use of the plant product in the
treatment of hepatitis.

**Aim**

To find out the relative hepatoprotective efficiency of the alcoholic
fraction of *Nigella sativa* L. Seeds and thymoquinone (active compound in
*Nigella sativa*).

**Objective**

To study the protective efficiency of the alcoholic fraction of *Nigella
sativa* L. seeds against D-galactosamine / Lipopolysaccharide induced
hepatitis.
To determine the antitoxic activity of the alcoholic fraction of the *Nigella sativa* L. seeds.

To prove the anti oxidant activity of the *Nigella sativa* L. seed extract.

To prove the acute antitoxic and hepatoprotective efficiency of thymoquinone (one of the active compounds of *Nigella sativa* L.).

To confirm the protective effect of the extract and thymoquinone in the ultrastructural changes of liver and kidney.

**Scope**

The present study would afford a scope for the clear basic understanding of the mode of action of *Nigella sativa* thus it can be utilized as a feed additive or as a therapeutic drug at the commercial level.