1.1. HEALTH AND FOOD

“Health” is a state of complete physical, mental and social well-being and not merely the absence of diseases or infirmity. Eating the proper kind of food in the right amount is essential to keep us healthy. In other words our health is dependent on our eating pattern.

The term food refers to anything which is nourishing to the body. Food is essential because it contains substances which perform important functions in our body. These essential substances contributed by our food are called nutrients. If these nutrients are not present in our food in sufficient amounts, the result is ill health and sickness (Swaminathan, 1996).

Food also contains many substances which are non-nutrients but make the food more palatable and attractive eg., tastemakers, colouring and flavoring agents in food. These “Food adjuncts” release the monotony of the staleness of food materials and increase the pleasure of eating. These are generally known as spices and condiments (Pruthi, 1992).

1.2. 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” (Lampe, 2003).

Spices are extracted from different plant parts such as rhizomes (ginger, turmeric), leaves (mints, tejpat), kernel (nutmeg), aril (mace), bark (cinnamon), bulbs (onion, garlic), floral parts (clove, saffron) fruits (cardamom, chillies), berries (pepper) and seeds (coriander, celery). Generally, spices are classified according to their 1. economic importance, 2. methods of cultivation, 3. the plant part or component used, 4. botanical families (Samba Murty and Subrahmanyam, 1989).
The importance of spices in our life are:-

i) Serve as appetizers, therefore they are also termed as ‘food adjuncts’ or ‘accessories’,

ii) Add a tang and flavour to insipid or bland food.

iii) Increase the secretion of saliva rich in ptyalin which facilitates food digestion

iv) Possess antioxidant properties,

v) Are used as preservatives in pickles and chutneys,

vi) Possess strong antimicrobial and antibiotic activities,

vii) Have medicinal value,

viii) Act as mouth freshener, cleans the oral cavity from adhesion and bacteria, also protects the mucous against thermic, mechanical and chemical irritation,

ix) Impart flavour to beverages, and

x) Ingredient of some cosmetics.

Given the wide range of botanical species and plant parts from which spices are derived, spices can contribute 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 (Samba Murty and Subrahmanyan, 1989).

1.3. CLOVE

Clove is the dried unopened flower bud of *Eugenia caryophyllata* (Synonym *Syzygium aromaticum*), a medium statured, cone-shaped, evergreen tree belonging to the family Myrtaceae. Clove tree attains a height of 10 to 12 meters. The stem is usually folked near its base with two or three main branches. Smaller branches are slender, rather brittle and
covered with grey bark. The leaves appearing in pairs, are lanceolate, acute at both ends and are of dark shining green colour. The aromatic nature of the leaves is due to numerous oil glands found on their undersurfaces. The flower buds are greenish when fresh and are borne on ends of the branches in small clusters. The unopened flower buds, which are picked green and dried in the sun till they become dark brown, form the 'clove' of commerce. The buds have slightly cylindrical base which is surmounted by the plump ball like unopened corolla which is surmounted by the four toothed calyx. If the bud is left unpicked, the flower develops after fertilization into a fleshy purple and one-seeded oval fruit as 'Mother of Clove'. The fruit is about 2.5 cm. long and 1.25 cm. in width. The seed is oblong, rather soft in texture and grooved on one side. The leaves, unripe fruit and broken clove, including the stalk are all aromatic and yield an essential oil (Samba Murty and Subrahmanyan, 1989).

1.3.1. CONSTITUENTS

The principal constituent of cloves is the volatile oil, of which they contain from 15 to 20%. They also contain gallotannic acid (13%) and a crystal body, ‘caryophyllin’, which, however, is odourless, and appears to be a phytosterol (Duke, 1999). The other constituents are listed in Table 1.

1.3.2. ACTION AND USES

Cloves are stimulating and carminative to the alimentary canal; they are used in flatulence, dyspepsia and as adjuvants to other medicines. The medicinal properties of cloves are resident principally in the volatile oil, which may be given on sugar, or in capsules with menthol, peppermint, or creosote. The oil is used to flavour emulsions; and is a good carminative to correct, the griping action of purgatives, 2 centimils (0.02 milliliters) being sufficient in each pill. Fresh infusion of the clove contains the astringent matter as well as some of the volatile oils; the infusion and the clove water are good vehicles for alkalies and
aromatics. Oil of the clove is a powerful antiseptic and preservative. It is applied to decayed teeth as a local anesthetic (Pandey and Anitha, 1988).

1.4. EUGENOL

Eugenol (C₆H₃C₃H₅OCH₃OH) is a phenol found in oil of cloves, oil of pimento, and other oils. It is official in the U.S. Pharmacopia. It may be obtained by shaking oil of cloves with excess of 5 or 10 per cent solution of sodium hydroxide, drawing off the resulting solution of Eugenol-sodium, washing it with ether and decomposing by means of diluted sulphuric acid. The Eugenol which separates is washed with solution of sodium bicarbonate and finally distilled with steam or in vacuo. It occurs as a colourless or slightly yellow optically inactive liquid, with an odour of cloves and pungent spicy taste. Specific gravity, 1.072 to 1.074. Boiling-point, 251⁰C to 253⁰C. It gives a blue color on the addition of solution of ferric chloride to its alcoholic solution; on oxidation with potassium permanganate it yields vanillin. Eugenol should be preserved in well-stopped bottles, protected from the light.

1.4.1. ACTIONS AND USES

Eugenol is an antiseptic and is not toxic. It has some local anesthetic properties and is a useful solvent of other local anaesthetics, such as pure cocaine, or erythrophloeine hydrochloride for use in dental practice. It is also used in combination with astringents as a mouth wash after tooth extraction. An antiseptic ointment of eugenol with hydrous wool fat has been used for ecozema. Eugenol is administered, in the same manner as oil of cloves in phthisis, as carminative and antiseptic.

1.5. LIVER AND THE LIFE

Liver, the largest organ of the body comprising 2-3% of the total adult body weight, is primarily concerned with the metabolic activities of the organisms. Hepatocytes (the liver cells) are metabolic superachievers in the body. They play critical roles in synthesizing molecules that are utilized elsewhere to support homeostasis, in converting molecules of one
type in to another and in regulatory energy balance (Sheila and Dooley, 1993). It is also the central site for the biotransformation of xenobiotics and therefore is involved in the detoxifying mechanism of the body. Liver is responsible for clearing the chemical toxins in the blood and in this process it is exposed to high concentration of toxicants and toxic metabolites making it very susceptible to injury (Glaister, 1986) infections, hepatic disfunctions and toxins are the major causes of hepatic injury (Kirsch et al., 1995). Nevertheless, liver is a very resilient organ and endures injury to a very great extent and new liver cells develop rapidly when needed, to replace damaged cells. Capacity for regeneration is considerable and damage is usually extensive before it is evident. The symptoms of hepatic injury are only manifested when regeneration of liver cells can not cope with the pace or magnitude of damage or when there is irreparable damage (Kirsch et al., 1995).

1.6. FOOD AND FEED MEDIATED TOXINS

One of the serious causes of severe hepatic injury is food and feed mediated toxins. Food and feed when stored under adverse conditions of moisture and temperature get invaded by some fungi. This is considered as the most common food and feed contamination. Some of these show toxic effects on animals and human beings. The main species of molds belong to the genera *Penicillium* and *Aspergillus* (Hesseltine et al., 1970 and Christensen, 1981).

In Russian literature there have been reports that fungal contaminated foods and feeds are the probable causative factors in certain human and animal diseases. As early as 1940, the first systematic studies were initiated in U.S.S.R. These related to stachylotryotoxicosis, primarily affecting horses was subsequently shown to be caused by a toxin in the etiology of disease in cattle and man (Sarkisov, 1947 as cited by Kirsch et al., 1995).

In the United States, the occurrence of certain diseases of unknown etiology in animals have been reported and these are thought to be the results of ingestion of mouldy feeds (Forgaes and Carll, 1962). It
will be interesting to know that in several districts of Japan, food and feed were collected from inhabitants and a parallel epidemiological study on the regional cancer mortality has been carried out for many years. The scientists speculated on the possibilities of fungal metabolites in yellowed rice being the causative factors in certain liver diseases in man (Kinosita and Shikat, 1965). (Table 2)

1.7. MYCOTOXINS, THE SECONDARY METABOLITES

During the invasions upon food and feed, the fungi utilize the food for its own primary metabolism which provides the organism with its energy, its synthetic intermediates and key macromolecules such as nucleic acids and proteins. There may be secondary reaction which can be termed as “secondary metabolism”, which involves biosynthetic processes leading to the formation of compounds known as “secondary metabolites” (Turner, 1971).

It has been recognized for many years that contaminated feed causes certain disease syndromes in animals and human beings. Probably, the toxic secondary metabolites are the causative factors whose functions in the etiology of diseases have been overloaded for many years (Kinosita and Shikat, 1965).

Some of the diseases were reproduced by feeding the fungal culture on various substrates (Sippel et al., 1953). Historically, biochemists and microbiologists have been aware of the secondary reactions. As a result of the observations, such as screening the secondary metabolites, antibiotics and mycotoxins have been isolated which had undesirable effects on the tissues of higher organisms (D’Mello and Mc Donald, 1997).

1.8. AFLATOXINS, THE MYCOTOXIN

Aflatoxins, the major group of mycotoxin were discovered in 1960 following the deaths of 100,000 young turkeys in England, and high incidences of liver disease in ducklings in Kenya and hatchery reared trout in the United States. English scientists soon established the cause of all these problems to be toxins produced by the common moulds
Aspergillus flavus and A. parasiticus. Assay techniques were devised and preliminary toxicological studies were carried out (Sargent et al., 1963).

Aflatoxins are named by letters and subscripts. Aflatoxin B₁, the most toxic compound, is usually associated with aflatoxin B₂: these compounds are usually formed by both A. flavus and A. parasiticus. Aflatoxins G₁ and G₂ are formed only by A. parasiticus (Klich and Pitt, 1988). Aflatoxins M₁ and M₂ are formed in milk when aflatoxin B₁ and G₁ are ingested through the feed.

Aflatoxins have both acute and chronic toxicity in animals, and produce four quite different effects: acute liver damage (Bhat and Krishnamachary, 1977), liver cirrhosis (Kirsch, et al., 1995), induction of tumours (Groopman et al., 1992) and cancer and other genetic effects (Smith and Moses, 1985).

1.9. HEPATOPROTECTION

The liver damage caused by pathogens as well as chemical agents are 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 (Neha and Rawal, 2000). No established therapy is available in allopathy for patients with alcoholic liver cirrhosis.

Supportive treatment with vitamin supplements and anti-inflammatory drugs like colchicines only help to prolong the life span in a very limited manner (Hurley and Horowitz, 1990). Even if alcohol-induced liver damage is diagnosed in the initial stage there is no therapy available for inducing faster recovery and again supportive therapy and abstinence from alcohol are the only alternatives available (Sherlock, 1990). Lack of allopathic medicines for the cure of hepatic injury exhorted the scientists to explore natural remedies.

1.10. CLOVE AND THE PRESENT STUDY

For the present investigation Eugenia caryophyllata (Synonym Syzygium aromaticum) has been selected. The obvious reason in choosing this spice in the present study as feed additive is multipurpose:-
➢ It is natural and therefore a healthier approach to enhance the detoxifying efficiency by protecting the liver without side effects (Susila Appadurai, 2001).

➢ It is well known for its flavour worldover and is accepted by common man as a spice and hence is a food additive (Lampe, 2003).

➢ It has been reported to posses a number of medicinal properties:- The antioxidant, antitumor, antibacterial, antimutagenic, hepatoprotective, antiviral, anti-inflammatory, anti aflatoxic, hypocholesterolemic, neuroprotective, etc. (Duke, 1999)

While administering any herbal product in the alternative systems of medicine, multiple herbal therapy is being practiced in Ayurveda and Sidda systems. In Homeopathy alcoholic extract (mother tincture) incorporated at various concentrations in sugar balls, is prescribed for treatment. But in the allopathy, purified single active principle is generally administered (Bown, 1995).

Hence, in the present investigation the entire flower buds, its alcoholic extract and one of the active principles, ‘eugenol’ of the bud have been administered to rats, in order to have a clear knowledge on the mode of action of this spice.

Screening of E. caryophyllata also for hepatic toxicity becomes mandatory for the present study. This is because in some of the herbal products (formulations that have been commercialized) hepatotoxicity has also been documented for many years (Chitturi and Farrell, 2000). 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).
1.11. **AIM**

To find out the relative hepatoprotective efficiency of the clove (flower bud), a particular fraction (alcoholic) of the clove and a pure compound “eugenol”, which is present in the clove.

1.12. **OBJECTIVE**

The present investigation was designed with the following objectives;

1. To study the alleviating effect of the clove against aflatoxicosis.
2. To determine the antitoxic activity of the alcoholic fraction of the clove.
3. To prove the acute antitoxic activity of eugenol (one of the components of the clove).
4. To compare the hepatoprotective efficiency of the clove, alcoholic fraction and pure eugenol with sylimarin (a proven hepatoprotective agent).

1.13. **SCOPE**

With the clear basic understanding of the mode of action of the clove, it can be utilized as a feed additive or as a therapeutic drug at the commercial level to protect the liver and hence the life.