CHAPTER 1

Introduction
In traditional societies, nutrition and health care are interconnected and many plants are consumed both as food and for medicinal purposes (Etkin, 1996; Pieroni, 2000). Different parts of plant such as leaves, flowers, stems and roots are used to prevent, relieve and treat illness. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, scientist has explored the medicinal properties of a large number of plants and their healing components have been extracted and analyzed (Raghu et al., 2011; Ramesh et al., 2011). Vincristine (an antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. Despite of modern medicines, many branches of science such as aromatherapy, homeopathy, naturopathy etc. also used plants for their specific medicinal properties.

World Health Organization (WHO) estimates 80% of the world populations presently use herbal medicine for some aspect of primary health care (WHO, 2008). There are over 750,000 plants on earth. Only around 1,000 of 365,000 known species of plants have been studied for their medicinal properties (Tabrez, 2011; Robinson and Zang, 2011). Several medicinal plants (Rasayana) have also been extensively used in the Indian traditional system of disease treatment (Ayurveda). Of all the plant foods, vegetable strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological system (Bajpai et al., 2005). Vegetables offer the maximum diversity. There are hundreds of varieties available in many different colors, shapes, sizes, flavors and textures.
1.1 Background history of vegetables

The written history of vegetables used in India starts from Vedic era. The disease preventive and health promoting approach of 'Ayurveda', which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic and finds increasing acceptability in many regions of the world. Ancient Ayurvedic physicians had developed certain dietary and therapeutic measures to arrest/delay ageing and rejuvenating whole functional dynamics of the body system. This revitalization and rejuvenation is known as the 'Rasayan chikitsa' (rejuvenation therapy). Traditionally, Rasayana drugs are used against many disorders with no pathophysiological connections according to modern medicine (Govindarajan et al., 2005).

Vegetables are edible plant or part of herbaceous plants which are our most important nutritious and vital foods. These are the indispensable ingredient of Indian food and present in the Indian cuisine since the Vedic era. Some vegetables mentioned in the Rigveda are the lotus stem (visa) and the cucumber (urvaruka). Vedas also refer to several others, like lotus roots (shaluka), bottle-gourd (alabu), water-chestnut (saphaka, mulali), two other aquatic plants (avaka and andika) and bitter gourd (karivrnta or kara-vella) (Charak and Dradhblala, 1996). The Buddhist and Jain canonical literature refers to yams (aluka), two convolvulus roots (etaluka and kadambu) and several leafy vegetables. Kautilya in his Arthashastra refers to the rajdhana or ksiri (now kauki, Manilkara kauki) and to the cucumber as chidbhita.

Most of the vegetables are mentioned in the Ramayana. The great epic speaks of the surana or elephant yam (vajrakanda), pindaluka (possibly the sweet potato), bottle gourd (kalasaku), sleshmataka and lasora (both Cordia species that bear fruits
which can be cooked or pickled), karira (Capparis decidua, with edible sour berries) (RSS feeds, 2009).

In Ayurveda, certain vegetables, such as brinjal, cucumber and radish reduce pitta and kapha. Vegetables in Indian food find their mention in Sanskrit and other literatures as well. Ancient literatures show the ways of preparation of many vegetables. After 1500 AD various vegetables of Indian food originated in foreign nations. These vegetables have become inseparable from the Indian cooking (Tripathi, 1998).

"Vegetable" comes from the Latin *vegetabilis* (animated) and from *vegetare* (enliven) which is derived from *vegetus* (active), in reference to the process of a plant growth. This in turn derives from the Proto-Indo-European base *weg-* or *wog-*, which is also the source of the English *wake*, meaning "become (or stay) alert. The word "vegetable" was first recorded in English in the 15th century (Swedenborg, 2003). The meaning of "vegetable" as "plant grown for food" was not established until the 18th century (Ayto, 1993).

1.1.1 Vegetable and nutrition: Vegetables are the primary source of food for human beings. It contains various constituents which are necessary for a human body to live. Vegetables are eaten in a variety of ways, as part of main meals and as snacks. The nutritional content of vegetables varies considerably; they contain protein, fat, vitamins, dietary minerals, fiber and carbohydrates (Woodruff, 1995; Wargovich, 2000). Vegetables contain a great variety of other phytochemicals, thus they contain valuable food ingredients which can be successfully utilized to build up and repair the body.
According to Ayurveda, dark green leafy vegetables have a special place in the daily diet, they are considered a particularly nutritious class of vegetables and modern science says they contain important minerals and vitamins. Leafy greens help to balance pitta and kapha. Leafy greens contain nutritious juices that help to replenish liquid in the system and purify the subtle channels of the body called shrotas. The natural juices and fibers help to purify and refresh the physiology (Thripathi, 1998).

1.1.2 Types of vegetables: There are different kinds of vegetables. They may be edible roots, stems, leaves, fruits and seeds. Each group contributes to diet in its own way (Robinson, 1990).

Leafy vegetables (such as collard greens and dark green lettuce) contain lots of water, carbohydrates, rich in carotenoids (precursors of vitamin A) and vitamin C. They are good sources of fiber, folate and supply varying amounts of iron and calcium. Dark-green leafy vegetables also contain high content of chlorophyll which is a natural blood purifier and anti-inflammatory. It also helps to bring acid/alkaline balance to the body, helps to remove unwanted residues and activate enzymes.

Flowers, buds and stalks (such as celery, broccoli, and cauliflower) tend to be rich in vitamin C, calcium and potassium. They are also a good source of fiber. Cauliflower and broccoli also provide cancer-fighting compounds.

Seeds and pods (such as lima beans, peas, and corn) generally have more plant protein than other vegetables. They are also good source of complex carbohydrates and contain varying amounts of B vitamins, zinc, potassium, magnesium, calcium and iron.
Roots, bulbs and tubers are also good sources of nutrients. Potatoes are a good source of vitamin C and potassium. Sweet potatoes and carrots are great sources of beta carotene. Radishes and turnips are good sources of fiber and vitamin C. Several studies suggest that onions and garlic may lower blood pressure and cholesterol levels.

Fruit vegetables (such as eggplants, squash, peppers, and tomatoes) are good sources of vitamin C.

1.1.3 Food Value of vegetables: Ayurveda recommends that you have some leafy greens each day to help meet the nutritional requirements for optimal health. The USDA dietary guidelines (USDA, 2000) encourage consumers to eat at least 2 servings of fruits and 3 servings of vegetables each day. In some countries, consumers are encouraged to eat up to 10 servings of fruits and vegetables per day (WHO, 2011).

1.1.4 Health benefits of vegetables: Vegetables not only quench hunger but apart from boosting the immunity, it helps to prevents symptoms of cancer, arthritis, builds up blood, gives energy, strengthens the metabolism and stimulates hormones. Vegetables contain various medicinal and therapeutic agents, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anti-carcinogenic properties (Gruda, 2005; Steinmetz and Potter, 1996). Vegetables also supply trace elements which are necessary for the human beings. Iodine, for instance, is essential for thyroid hormone which regulates physical and mental activities, cobalt for increasing the number of blood corpuscles and zinc for proper growth.

Vegetables are known to have substantial amounts of natural antioxidants that neutralize free radicals that are harmful to the body. Cell damage is something that we
cannot avoid due to the high levels of pollution that we are exposed to everyday. The antioxidant activity of vegetables is regarded worldwide as an important area of the nutritional and phytotherapeutic research (Karakaya et al., 2001; Ogle et al., 2003; Sreeramula and Raghunath, 2010; Molan et al., 2012).

1.2 Antioxidants

An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate (Halliwell, 1995). These are nutrients (vitamins and minerals) as well as enzymes (proteins in body that assist in chemical reactions) (Mandal et al., 2009). They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, stroke, alzheimer's disease, rheumatoid arthritis and cataracts (Maxwell, 1995; Halliwell, 2000; Young and Woodside, 2001).

The anti-oxidative system includes both enzymatic and non-enzymatic systems. The non enzymatic system includes ascorbic acid (vitamin C), α-tocopherol, carotenoids etc. and enzymatic system include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and polyphenol oxidase (PPO) etc.

1.3 Free Radicals

Free radicals are chemical compounds that have one or more unpaired electrons which are highly reactive. If free radicals are not inactivated, a sequence of free radical generation is started (Fig. 1.1) and their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. These free radicals are produced in living system by endogenous and exogenous sources. Endogenous sources are many physiological processes including
oxygen metabolism, phagocytosis, apoptosis or coagulation and exogenous sources include cigarette smoke, drugs, pesticides, ozone, nitrogen oxides, sulfur dioxide, car exhaust, x-rays and UV light etc. (Halliwell, 2007; Lien et al., 2008).

Fig. 1.1 Sequence of free radical generation and the role of antioxidants (Bulger and Helton, 1998).

1.3.1 Free radical and tissue damage: The most important free radicals in the body are the radical derivatives of oxygen better known as reactive oxygen species (ROS) (Halliwell et al., 1995; Halliwell, 2007). It is generally accepted that reactive oxygen species (ROS) and/or free radicals play an important role in the development of tissue damage and pathological events in living organisms (Kehrer, 1993; Halliwell and Gutteridge, 1999). Free oxygen radicals play a role in the etiology of several diseases like arthritis, cancer, atherosclerosis etc. The oxidative damage to DNA may play vital role in aging and the presence of intracellular oxygen also can be responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause damage to critical cell biomolecules.
DNA damage is an extremely common event in all living cells. There are multiple ways in which cellular DNA can be altered from its structural integrity. A number of intrinsic and extrinsic factors induce structural changes that could lead to alteration in the coding property of DNA and cellular toxicity. However, organism has also evolved many intricate mechanisms to detect and repair this damage in DNA. Essentially, in mammalian cells, there are four major DNA repair pathways:

(1) A simple reversal of the damage: *i.e.* methylation of guanine can take place by an enzyme like O6-methyl guanine methyl 1 transferase which removes the methyl group and avoid the possibility of mismatch formation. Monomerization of pyrimidine dimers is catalyzed by polymerase enzyme, dependent upon light of wavelength above 300 nm.

(2) Nucleotide excision repair (NER): Inner and intra-strand cross links and inter-strand adduct formation with some drugs and antibiotic lead to major distortion in the structure of DNA, affecting its normal information transfer function. NER involves four major steps (a) recognition of damage (b) removal of damaged portion of the DNA strands (c) filling up of gap created by appropriate DNA polymerase using the other strand as a template (d) ligation of the newly synthesized strand with downstream sequence. NER also includes mismatch repair using the other strand as template.

(3) Base excision repair (BER): This mode of repair is a simple one and confined to a base. Alkylation, spontaneous deamination and loss of bases can cause mutation. All these events are induced by free radical interactions with bases. BER is the pathway that has a close link to the free radical metabolism. This pathway consists of four steps and can be divided into two sub path way; Long patch (to fill up to 13
nucleotide) and short patch (to fill a single nucleotide gap). Step 1, altered base is recognized and cleaved from the sugar phosphate moiety by DNA polymerase. Step 2, at the same time AP endonuclease (APE1) attaches itself to the 5’ side of the base to break the chain. Step 3, the pol β fills up the one nucleotide gap and also release the 5’ deoxyribose phosphate and DNA ligase III – XRCC1 (X-ray repair cross complimenting gene 1) arrives at the site. Step 4, DNA ligase III sealing the nick and pol β dissociating from the site.

(4) recombinant repair including the end joining (RR): Recombinant repair is a mode of DNA repair that is most prevalent in rapidly dividing tissues/cells, where the damaged or mutated portion of DNA is removed or replaced with a homologous portion from a sister DNA molecule (Rao, 2009).

Free radical induced DNA damage could be deleterious, if not repaired or the balance between oxidative damage and repair mechanism of DNA is disturbed by generation of excess free radical, lead to cellular injury and finally death. Stress plays a potential role in liver diseases viz., hepatic fibrosis and cirrhosis (Parolo and Rabino, 2001). A number of xenobiotics generate reactive oxygen species which react with cell membrane and induce lipid peroxidation or cause inflammation, tissue damage in liver. In the living systems, liver is considered to be highly sensitive to toxic agents such as drugs, food additives etc. There are so many drugs available in the market that’s overdose induce hepato-toxicity, acetaminophen or paracetamol (PAR) is one of them.

Acetaminophen is the most commonly used drugs for treating pain and fever (Kaufman et al., 2002). It is metabolized primarily by glucuronidation and sulfation. These major conjugates PAR- sulfate (PAR-SULF) and PAR-glucuronide (PAR-
GLUC), being more water soluble than the parent compound, are elimination from the liver and blood mainly via urine (both) and a little via bile (PAR-GLUC) (Tone et al., 1990). A small amount of PAR is probably metabolized via a third metabolic pathway, that is oxidation by the microsomal cytochrome P450 (CYP-containing mixed function oxidase system (MFO) to NAPQI. A glutathione 1,4-Michael adduct of NAPQI and the corresponding cystein conjugate and mercapturic acid breakdown products were found in human urine after ingestion of PAR (Prescott, 1980).

Liver disease remains as one of the serious health problem and there are not any satisfactory liver protective drugs in allopathic medicine system. Thus, much attention has been focused on the investigation of herbal drugs in the management of various liver disorders. The bioactive compounds or phytochemicals such as secondary metabolites of plant origin has been reported for their potential health beneficial role that include, anti microbial, anti-carcinogenic, anti-inflammatory, anti-allergic, hepatoprotective etc. (Shahidi and Wanasundra, 1992; Rice-Evans et al., 1996).

1.4 Phytochemicals from vegetables

Nature provides a lot of plant chemicals or phytochemicals which exert multiple biological effects due to their antioxidant and free radical scavenging abilities as well as protective action against different chronic diseases (Craig and Beck, 1999; Choi et al., 2002). These phytochemicals are naturally present in essentially all plants. Various classes of phytochemicals have been shown to have antioxidant property. They have different mechanism of action and act at different sites in the chain reaction. The activity of natural product as an antioxidant is due to the presence of substituted groups such as carbonyl, phenolic, phytol side chain etc.
They may be phenolic or non phenolic groups. The hydroxyl group donates hydrogen to radicals which are converted to a stable non-radical product and the chain propagation is terminated. Phenolic group at para position enhances the antioxidant property. Non-phenolic compounds participate in the antioxidant mechanism through electron transfer and resonance stabilization process. The quinine acts as an electron acceptor and prevents free radical chain reaction.

Phenolic compounds or polyphenols constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, with more than 8000 phenolic structures currently known. Natural polyphenols can range from simple molecules (quinones, phenolic acids) to highly polymerised compounds (lignins, melanins, tannins) with flavonoids such as flavonols, flavones, isoflavones, flavonones, flavanols and anthocyanins representing the most common and widely distributed sub-group (Bravo, 1998).

Phenolics are therefore an integral part of the diet, with significant amounts being reported in vegetables, fruits, tea and traditional plants (Bahorun et al., 2004; Luximon et al., 2005). Although the dietary intake of phenolics varies considerably among geographic regions, it is estimated that daily intake range from about 20 mg to 1 g which is higher than that for Vitamin E (Hollman and Katan, 1998). The effectiveness of a dietary antioxidant will depend on a number of factors, how and where they are being generated and the accessibility of the antioxidant to possible sites of damage (Morton et al., 2000). Many phenolic compounds have been shown to have antioxidant activity in vitro (Shahidi and Wanasundara, 1992) and reduce the risk of diseases.
Epidemiological studies showed that the consumption of vegetables reduce the risk of various pathological events such as cancer (Steinmetz and Potter, 1996), cardio- and cerebro-vascular diseases (Rimm et al., 1996), Alzheimer's disease, rheumatoid arthritis, cataracts as well as ageing. They may also have stimulating or regenerating effects on hepatocytes and restore the protective activities of living system through their antioxidant properties such as vitamin C, vitamin E, carotenoids, lycopenes and flavonoids (Steinberg, 1991; Willett, 1994; Sumazian et al., 2010; Olajire and Azeez, 2011).

Thus, the antioxidant activity and nutraceutical value of vegetables is an important area of the nutritional and phytotherapeutic research worldwide. Vegetables have also attracted a great deal of interest as physiologically functional foods and as a source for the development of its constituents as drug candidates because of low cost and wide availability.

Keeping in view the above background, systematic biochemical approaches are necessary to study the role of antioxidants in the protection of DNA damage and hepatoprotective activity of phytochemicals found in vegetables. The proposed study is expected to scientifically validate new information on the utility of various vegetables to be used as therapeutic agents for prevention and management of many diseases with special reference to hepatotoxicity and DNA damage.

1.5 Aims and objectives

The aim of the present work was to evaluate the antioxidant activity of some promising varieties of commonly consumed vegetables in North India, in relation to their phenolic content and their *in vitro* hepatoprotective activity against reactive
oxygen species (ROS) by investigating antioxidant enzymes (AOE). The role of extracts was also ascertained in protection of DNA damage by ROS.

Objectives

1. To identify the vegetable varieties with high antioxidant and free radical scavenging activities by screening for active biomolecules.

2. To determine important phytochemicals from selected promising varieties.

3. To study the role of extracts in protection of DNA damage induced by reactive oxygen species (ROS).

4. To study the hepatoprotective effects of the extracts under *in vitro* conditions.