2. REVIEW OF LITERATURE

2.1. NUTRACEUTICALS

About 2000 years ago, Hippocrates correctly emphasized “Let food be your medicine and medicine be your food.” Currently there is an increased global interest due to the recognition that “nutraceuticals” play a major role in health enhancement. The term “Nutraceutical” was coined by combining the terms Nutrition and Pharmaceutical in 1989 by Dr. Stephen De Felice, chairman of the foundation for innovation in Medicine (Brower 1999).

A nutraceutical is any non-toxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention (Dillard and German 2000). According to Hasler (2000), functional foods can be defined as “those providing health benefits beyond basic nutrition and include whole, enriched or enhanced foods which have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels. In Japan, a related terminology and concept are foods for specified health uses” (FOSHU). The first FOSHU product – hypoallergenic rice was approved in 1993. According to the Nutrition Improvement Law, FOSHUs are defined as “foods in the case of which specified effects contributing to maintain health can be expected based on the available data concerning the relationship between the foods/food contents and health, as well as foods with permitted labelling which indicates the consumer can expect certain health effects upon intake of these particular compounds” (Arai et. al. 2001).

Nutraceuticals may range from isolated nutrients and dietary supplements to genetically engineered “designer” foods, herbal products and processed products such as cereals, soups and beverages. Some examples of nutritive nutraceuticals or “functional food ingredients” are dietary fiber, polyunsaturated fatty acids (PUFA, fish oil), proteins, peptides, amino acids, keto acids, minerals, antioxidative vitamins and other antioxidants (glutathione, selenium, etc) (Andlauer and Fürst 2002; Kruger and Mann 2003). PUFAs, especially the n-3 fatty acid family, are claimed to exert a protective effect against the development of cardiovascular and inflammatory diseases (Fürst and Kuhn 2000; Fang et. al. 2002). Epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of cardiovascular diseases.
(Bazzano et. al. 2001) and certain forms of cancer (Liu 2003). Although the protective
effects have been primarily attributed to well-known antioxidants, such as ascorbic acid,
tocopherols and b-carotene, plant phenolics may also play a significant role (Soobrattee
et. al. 2005).

Nutritional therapy is a healing system using dietary therapeutics or nutraceuticals as a
complementary therapy. This therapy is based on the belief that foods can not only be
sources of nutrients and energy but could also provide medicinal benefits. In other words,
foods can be medicine if they were properly prepared. Both a long folk history of foods,
along with modern scientific research, continue to extend the idea about functional foods
or nutraceuticals. Nutritional therapy mainly uses functional food, nutraceuticals, and
dietary supplements to promote the body’s natural healing based on knowledge from food
sciences, clinical nutrition studies, and epidemiological studies (Thomson 2006).

According to nutraceutical and nutritional therapy theory, it achieves this goal by using
efficacy of such nutraceuticals in detoxifying the body, avoiding vitamin and mineral
deficiencies, and restoring healthy digestion and dietary habit. Although many health
problems require specific medications, many other conditions such as some degenerative
and chronic diseases can be relieved alternatively with nutritional therapy. These
disorders may range from chronic fatigue, energy loss, insomnia, and osteoarthritis, to
backache, skin complaints, and asthma (Berger and Shenkin 2006).

2.1.1. Overview of Plants

Herbs and spices, traditionally well-known sources of nutraceuticals, are being used
today at a rate of 860 million lb per year in the United States alone. The Food and Drug
Administration now permits b-glucan, at a 0.75g serving, to carry the claim that it
reduces the risk of heart disease. These developments notwithstanding, the growth of
nutraceutical foods has been hampered by their nonpatentability and, thus, their poor
right to be claimed as intellectual property. Almost anything in this field is prior art and is
considered to exist in the public domain (Shukla 1998).

Flavonoids are widely distributed in onion, endives, cruciferous vegetables, black grapes,
red wine, grapefruits, apples, cherries and berries (Hollman et. al. 1996). Flavanoids in
plants available as flavones (containing the flavonoid apigenin found in chamomile);
flavanones (hesperidin - citrus fruits; silybin- milk thistle flavonols (tea: quercetin,
kaempferol and rutin grapefruit; rutin buckwheat; ginkgo flavonglycosides - ginkgo), (Majoa et. al. 2005) play a major role in curing the cardiovascular diseases (Cook and Samman 1996; Hollman et. al. 1999). Flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure; by blocking the "suicide" enzyme cyclooxygenase that breaks down prostaglandins, they prevent platelet stickiness and hence platelet aggregation. Flavonoids also protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. Flavonoids block the enzymes that produce estrogen, thus reducing the risk of estrogen-induced cancers.

2.1.2. Uttarakhand Medicinal Plants

The ethnobotany and ubiquitous plants provide a rich resource for natural drug research and development. In recent years, the use of traditional medicine information on plant research received considerable interest (Garg et. al. 2007). The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. Considerable works have been done on these plants to treat cancer, allergies, inflammatory reactions etc (Pandey and Madhuri 2009). India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed awe-inspiring (Garg et. al. 2007). Over the past decade, herbal medicines have been accepted universally, and they have an impact on both world health and international trade. Hence, medicinal plants continue play an important role in the healthcare system of a large number of the world’s population (Akerele 1988).

Awareness about natural sources is worldwide on rise and in the context, and then novel biomolecules from natural products (plant sources) offer a great scope (Newman et. al. 2000) India being one of the 12 major diversities centers, hold large reservoir of plant genetic diversity, which can provide novel biomolecules. Ayurveda, Siddha and Unani systems of medicines offer a good base for scientific exploration of medicinally important molecules from nature. While the Western use of such information came under increasing scrutiny and the national and indigenous rights on these resources became acknowledged by most academic and industrial researchers. Meanwhile, the need for basic scientific investigations on medicinal plants using indigenous medical systems
has become imminent. Most recently emerging approaches such as advanced genomics has significantly facilitated the lead compounds for drug development, high throughput screening combinatorial chemistry with biology and computer assisted de novo drug design.

The reason why plants are best suited for insulating novel chemical entities arises from the fact that plant molecules have always shown compatibility through natural resistance or tolerance against them because of that approximately 60% of the anticancer and anti infective agents that are commercially available are of natural product origin (Cragg et. al. 1993) There seems to be increasing possibility of finding biological activity among plants with recorded medicinal uses rather than from plants randomly selected (Unander et. al. 1995).

**Types of Medicinal Plants**

A vast diversity exists among the Medicinal plants, having different properties. Some of these plants are described as below:-

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Family</th>
<th>Distribution</th>
<th>Part used</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Marenta arundinacea</em></td>
<td>Marantaceae</td>
<td>Cultivated throughout India.</td>
<td>Underground rhizome</td>
<td>Starch obtained from rhizome is astringent, sweet refrigerator and tonic.</td>
</tr>
<tr>
<td><em>Marsilea quadrifolia</em></td>
<td>Marsileaceae</td>
<td>Throughout India, in marshy places and along the banks canals.</td>
<td>Whole plant</td>
<td>The plant is sweet, astringent, acrid, anodyne, hypotonic, ophthalmic and diuretic.</td>
</tr>
<tr>
<td><em>Melia azedarach</em></td>
<td>Meliaceae</td>
<td>Throughout India upto</td>
<td>Roots, leaves and seeds.</td>
<td>The roots are acrid, mildy</td>
</tr>
</tbody>
</table>
### Nutraceutical evaluation of edible plants Colocasia esculenta and Girardinia heterophylla from high altitude

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Habitat Description</th>
<th>Part Used</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mentha arvensis</strong></td>
<td>Lamiaceae</td>
<td>Western Himalayas, throughout India.</td>
<td>Whole plant</td>
<td>The leaves are acrid, aromatic, thermogenic, stimulated and antiseptic.</td>
</tr>
<tr>
<td><strong>Merremia emarginata</strong></td>
<td>Convolvulaceae</td>
<td>Throughout India up to 900m.</td>
<td>Whole plant</td>
<td>The plant is bitter, acrid, diuretic. Useful in nephropathy, uropathy and rat bite.</td>
</tr>
<tr>
<td><strong>Merremia Tridentate</strong></td>
<td>Convolvulaceae</td>
<td>Throughout India on hedges and open waste land.</td>
<td>Whole plant</td>
<td>The plant is bitter astringent, used in inflammation and uropathy.</td>
</tr>
<tr>
<td><strong>Mesua nagassarium</strong></td>
<td>Clusiaceae</td>
<td>Throughout India, in evergreen forests up to 1,500m.</td>
<td>Flower and oil</td>
<td>The flowers are astringent, bitter, acrid and mildy. The uses are in vitiated condition of pitta, asthma and cough.</td>
</tr>
<tr>
<td><strong>Michelia champaca</strong></td>
<td>Magnoliaceae</td>
<td>Throughout India in evergreen forests and</td>
<td>Whole plant</td>
<td>The root bark and roots are purgative and emmenengeue.</td>
</tr>
</tbody>
</table>
Nutraceutical evaluation of edible plants Colocasia esculenta and Girardinia heterophylla from high altitude

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Vernacular Name</th>
<th>Major constitute</th>
<th>Medicinal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alchemilla vulgaris L.</td>
<td>Lady’s mantle</td>
<td>Tannins, flavonoids, salicylic acid (Chevallier 1996).</td>
<td>Treat mild diarrhea; as a wound healer, relieves menstrual cramps, improve regular cycle.</td>
</tr>
<tr>
<td>Mimosa pudica</td>
<td>Mimosaceae</td>
<td>Throughout India in hot and moist locality. Roots and leaves</td>
<td>The roots are bitter and astringents, acrid, and used in ulcer, dysentery and inflammation.</td>
</tr>
<tr>
<td>Mimusops elengi</td>
<td>Sapotaceae</td>
<td>Throughout India, South India, and Andaman Island. Bark and Flower</td>
<td>The bark, flowers and fruits are acrid, astringent, cooling, and antihelminthic, the fruits are used as a masticatory and help to fix loose teeth.</td>
</tr>
<tr>
<td><strong>Allium cepa</strong></td>
<td>Onion</td>
<td>Thiamin, riboflavin, beta-carotene, ascorbic acid (Jaume et. al. 1994).</td>
<td>Relives in intestinal gas pain, reduce hypertension, inflammation.</td>
</tr>
<tr>
<td>----------------</td>
<td>-------</td>
<td>---------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>A. schoenoprasum</strong></td>
<td>Chives</td>
<td>Alliin, sulphoxide, linoleic acid.</td>
<td>Used as heart and blood circulation remedies.</td>
</tr>
<tr>
<td><strong>Alnus crispus</strong></td>
<td>Alder</td>
<td>Tannins, oils, resin, beta-sitosterol (Duke 1985a).</td>
<td>As an astringent, with homeostatic function, inflammation.</td>
</tr>
<tr>
<td><strong>Aloe vera(L) Burm.</strong></td>
<td>Aloe</td>
<td>Aloin, aloeresin, anthraquinone glycoside (Cavallinin et. al. 2001).</td>
<td>Purgative, juice from leaves used for cuts and possibly other skin problems.</td>
</tr>
</tbody>
</table>

### 2.1.3. Wild Edible Plants

Wild edible plants have played an important role in human life since time immemorial. In India most rural inhabitants depend on the wild edible plants to meet their additional food requirements. The diversity in wild plant species offers variety in family diet and contributes to household food security. Today, most human plant food is based on rather limited number of crops, but it is clear that in many parts of the world the use of wild plants is not negligible (Prescott-Allen and Prescott-Allen 1990; Scherrer et. al. 2005; Bussmann et. al. 2006; Bussmann and Sharon 2006; Kunwar et. al. 2006; Cavender 2006; Pieroni et. al. 2007). Sometimes the nutritional value of traditional wild plants is higher than several known common vegetables and fruits (Nordeide et. al. 1996; Sundriyal and Sundriyal 2001; Orech et. al. 2007a). The Garhwal Himalaya region is the land of many beautiful holy places, valleys and hills. Most of the people of the Garhwal live in the villages. The area forms the middle and outer part of the Himalaya, which is rich in natural resources of which plant resources are prevalent. The forest resource plays an important role in the livelihood of the local communities. The rich plant diversity of...
the area is utilized by the local inhabitants in various forms as medicine, food, fodder, fuel, timber, agricultural implements, etc. Among these, wild edible plants play an important role in food supplement during scarcity for local inhabitants. Because of small land holdings and subsistence agriculture, the local people collect many wild edible plants for food. Many works have emphasized on the diversity and traditional uses of wild plants from this part of country (Gaur 1977; Gaur and Semwal 1983; Negi 1988; Negi and Gaur 1991, 1994; Samant and Dhar 1997; Maikhuri et. al. 2000; Kala 2007; Dhyani et. al. 2007). Although much has been documented on ethnomedicinal and floristic aspects of plants of this area, little has been reported about the wild edible plant resources of Srinagar and its adjacent area.

2.1.4. Wild Edible Plants of Uttarakhand Himalaya

Himalayas are known to be accretion of innumerous wildly growing plants having immense support towards dietary constitution of the local inhabitants. Wild edible leaves are frequently consumed throughout the various months and seasons of the year and gathered by the local inhabitants from high altitudinal zones of Nanda Devi Biosphere Reserve (NDBR). In addition to fresh consumption in season, many leaves are processed, fermented for storage and used off seasonally. Although, adults are familiar with various ethno biological uses and medicinal properties of the herbs, the younger generation is less aware regarding their uses as well as habitats. Wild edibles are of particular value for sustaining over long winters when resources from agriculture and others are scarce.

Uttaranchal (200 26’ and 310 38’ N latitude and 770 49’ and 800 6’ E longitude) covering an area of 53,483 sq. km and with the population density of 159 persons/m2 is paradise of wild edibles. The diversity of topographical and climatic conditions has favored the luxuriant growth of forests. Uttaranchal in general and Garhwal in particular are rich in its ethno-cultural, traditional and biological diversity. Nanda Devi Biosphere Reserve (NDBR), a world heritage site, occupies a special place in the biosphere reserve system of higher Himalayan region of India. The reserve is located in the northern part of western Himalaya with a core zone and two buffer zones. The people inhabiting the villages of the biosphere reserve traditionally use various plant parts as medicines, food, fodder, fuel, agriculture tools, building fibers, religious and other purposes. A total of 97 species are being utilized by the local people. Out of these 17 species are used as
Higher Himalayans are going through the phase of transition due to increasing population pressure, tourism related activities while; its rich herbal wealth is in huge market demand (Maikhuri et. al. 1998a). Besides, harvesting crops after tough hustle in their small and terraced fields and still being paid with low productivity local inhabitants are frequent enough to collect these wild edibles for food and other plants from their natural habitats to meet their subsistence requirements. Use of wild edibles as a supplement in the delicious indigenous cuisine of ethnic Bhotiya tribes is promising (Maikhuri et. al. 2001). Wild leaves other than fruits are among the most widely consumed wild edibles by the tribal community. Most of these plant species sprout out after the snow melts in the valley. Localities consume these leaves by preparing several recepies from them. There are almost 10-15 green leaved plants in the forests of biosphere reserve of Niti valley near to Indo-China border which are used by the tribal community in their traditional cuisine in one way or other. Some commonly eaten green vegetables of the area are fine to consume during winter season as they help in maintaining the body temperature. While, few of plants consumed for their leaves may be high in their fat content, others are rich in proteins; most of them are good sources of multi vitamins and minerals (Sundriyal 2003).

These wild green leafy vegetables necessitate a comprehensive field investigation with the prop up of laboratory data related to their nutritional and other essential dietary content, so that the habitat of economically important species can be conserved and if possible can be promoted for their domestication. Wild plants are all those gathered in the form of edible plant parts eg. roots, tubers shoots, leaves, twigs, flowers, fruits, fronds, bark, piths, buds and other vegetative parts (Samant and Dhar 1997). A very large section of the population in Garhwal Himalayas living close to the natural forest boundaries, particularly in remote and far flung valleys, depend upon a variety of wild plants for their subsistence (Sundriyal and Sharma 1996).
2.1.5. Wild Edibles as Source of Antioxidants

Consumption of fruits and vegetables is known to lower risk of several oxidative stresses, including cardiovascular diseases, cancer and stroke (Willett 2002) and such health benefits are mainly ascribed to phytochemicals such as polyphenols, carotenoids and vitamin C (Steinmetz and Potter 1996). Of these phytochemicals, polyphenols are largely recognized as anti-inflammatory, antiviral, antimicrobial and antioxidant agents (Narayana et. al. 2001). In the Indian Himalayan Region (IHR) over 675 wild edibles are known (Samant and Dhar 1997) of which Myrica esculenta Buch.- Ham. ex D. Don (family Myricaceae), commonly known as ‘Kaphal’, is amongst highly valued wild edible fruits growing between 900 and 2100m above sea level (asl). Species is distributed from Ravi eastward to Assam, Khasi, Jaintia, Naga and Lushi hills and extends to Malaya, Singapore, China and Japan (Osmaston 1927). It is popular among local inhabitants for its delicious fruits and processed products (Bhatt et. al. 2000). This species broadly resembles with Myrica rubra, found commonly in China and Japan. However, M. esculenta contains smaller fruits of around 4–5mm as compared with 12–15mm fruits of M. rubra (Gupta 1989). While information is available on phenolic contents, flavonoids, anthocyanins and antioxidant activity of M. rubra fruit extract, juice, jam and pomace (Bao et. al. 2005; Fang et. al. 2007; Zhang et. al. 2008; Fang et. al. 2009 and Zhou et. al. 2009).

2.1.6. Medicinal Properties of Wild Edibles

India has one of the largest concentrations of tribal population in the world. The forest plays a vital role in the economy as well as daily needs of the tribals. In times of scarcity when the staple food is in short of supply tribals collect many types of wild roots and tubers to supplement their meagre food available at home (Vidyarthi 1987). Nature has endowed plants with the genetic capacity to synthesize substances that are toxic and thus to ensure their survival against predators whether they be insects, fungi or animals including humans. Humans have learnt which foods are safe to eat or how such foods can be treated in order to destroy their toxicity. Some wild yams like Dioscorea alata, D. bulbifera, D. esculenta, D. oppositifolia, D. pentaphylla, D. tomentosa and D. wallichi are used as food by the tribal Kanikkars of Kanyakumari district in times of food shortage but only after extensive detoxification processes. D. alata is recommended to diabetic
patients as tolerable energy resources. The toxic principles of *Dioscorea* exhibit medicinal properties.

There have been studies to show that Dioscorea has antioxidant activities (Araghiniknam *et. al.* 1996) and the anti-inflammatory activity can be linked to the antiphlogistic effect of the steroidal saponins.

Some species of *Dioscorea* show antibacterial activity against Gram-positive bacteria and Gram-negative *Escherichia coli*, though specifically for *D. sylvatica* and *D. dregeana* (Kelmanson *et. al.* 2000). The plant is also cited with cardiovascular activity, hepatic effects and hormonal or oestrogenic effects.

Widely prescribed cortisones and hydrocortisones were indirect products of the genus *Dioscorea*. They are used for Addison’s disease, some allergies, bursitis, contact dermatitis, psoriasis, rheumatoid arthritis, sciatica, brown recluse spider bites, insect stings, and other diseases and ailments (Foster and Duke 1990).

Numerous studies have shown that yams are sources of diverse nutrients and non-nutrient molecules, many of which display bioactive properties. Some examples of such non-nutrient molecules in yam tubers are organic acids and polyphenols. Organic acids are widely distributed in fruits and vegetables. The content of organic acids in food not only influences their flavor, but also their stability, nutrition, and acceptability (Poyrazoglu *et. al.* 2002). Holloway, Argall, Jealous, Lee and Bradbury (1989) have reported the principal organic acids in some common root crops, including yams. Phenolic compounds, widely existing in plants, are important for their contribution to colour, sensory attributes and nutritional and antioxidant properties of foods (Maga 1978). Phenolic compounds are reported to have multiple biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties (Shui and Leong 2002). Yam tubers show strong enzymatic browning reactions when cut and exposed to the air. This browning has been attributed to the oxidation of phenolic compounds (Ozo *et. al.* 1984).
### 2.1.7. High Altitude Plants

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Family</th>
<th>Part used</th>
<th>Distribution and Uses</th>
<th>Altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Swertia chirata</em> (chiayata)</td>
<td>Gentianaceae</td>
<td>Whole plant</td>
<td>Found in Himalayas region. Used in antipyretic, hyperexia, fever.</td>
<td>1200–3000</td>
</tr>
<tr>
<td><em>Sassurea lappa</em> (kuth)</td>
<td>Asteraceae</td>
<td>Root</td>
<td>Found in Himalayas in alpine habitat.</td>
<td>2500–4000</td>
</tr>
<tr>
<td><em>Picorhiza kurroa</em> (kutkin)</td>
<td>Asteraceae</td>
<td>Root</td>
<td>Found in Himalayas region. Inflammatory process in liver, antioxidants, antiallergic.</td>
<td>3000–4000</td>
</tr>
<tr>
<td><em>Asparagus racemosus</em> (Shatavari)</td>
<td>Litiaceae</td>
<td>Whole plant</td>
<td>Throughout Himalayas. Used as antioxidants, dyspia, diarrhea, dysentery.</td>
<td>2000–3000</td>
</tr>
<tr>
<td><em>Hyoscyamus niger</em> (Indian henbane)</td>
<td>Solanaceae</td>
<td>Whole plant</td>
<td>Found in himalayan region. Used in hypertension,</td>
<td>1500–3000</td>
</tr>
</tbody>
</table>
2.1.8. Low Altitude Plants

The low altitude region is kumaon region comprising the districts Almora, Bageshwar, Champawat and Nanital.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Vernacular Name</th>
<th>Family</th>
<th>Distribution and properties.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abelmoschus crinatus</em></td>
<td>Kapsua</td>
<td>Malvaceae</td>
<td>Mucilage from stem, fiber is used as a thicking agent in the preparation of delicious sweet dish from rice flour.</td>
</tr>
<tr>
<td><em>Abrus precatorius</em></td>
<td>Ratti</td>
<td>Papilionaceae</td>
<td>Seed are inserted within eyes to use as a measure of weight for precious metals and stones.</td>
</tr>
<tr>
<td><em>Acacia Arabica</em></td>
<td>Khair</td>
<td>Mimosaceae</td>
<td>Agriculture implements particularly plough is made from wood.</td>
</tr>
<tr>
<td><em>Aegle marmelos</em></td>
<td>Bel</td>
<td>Rulaceae</td>
<td>The pulp of fruit is used as a refreshing drink. Leaves particularly are offered to lord SHIVA.</td>
</tr>
<tr>
<td><strong>Aesculs indica</strong></td>
<td>Pangar</td>
<td>Hippocastanaceae</td>
<td>The embryo is eaten by local people. Leaves are used as green fodder.</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------</td>
<td>-----------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td><strong>Agave Americana</strong></td>
<td>Rambans</td>
<td>Agavaceae</td>
<td>Leaf extracts are used for washing clothes. Fiber for cortage nets, bags.</td>
</tr>
<tr>
<td><strong>Bombax ceiba</strong></td>
<td>Semal</td>
<td>Bombabceae</td>
<td>Eaten by native children, glycine max (bhatt) given to cattle for skin diseases.</td>
</tr>
<tr>
<td><strong>Brassica compestris</strong></td>
<td>Sarson</td>
<td>Brassicaceae</td>
<td>Leaves are used as green vegetables, mustard oil extracts from seeds in cooking.</td>
</tr>
<tr>
<td><strong>Cannabis sativa</strong></td>
<td>Bhang</td>
<td>Cannabaceae</td>
<td>Bast fiber are used for unmaking cords and sacks.</td>
</tr>
<tr>
<td><strong>Centella asitica</strong></td>
<td>Brahmi</td>
<td>Apiaceae</td>
<td>Leaves are used as a brain tonic.</td>
</tr>
</tbody>
</table>

(www.Uttaranchal.org.uk/kumanplants.php)
2.2. FREE RADICALS

Free radicals are atoms or molecules which contain unpaired electrons. Since electrons have a very strong tendency to exist in a paired rather than an unpaired state, free radicals indiscriminately pick up electrons from other atoms, which in turn converts those other atoms into secondary free radicals, thus setting up a chain reaction which can cause substantial biological damage (Halliwell 1994).

A molecule becomes a free radical by accepting an additional electron or by losing an electron.

Accepting an electron: Reduction of molecular O$_2$ to the super oxide anion radical (O$_2^-$)

\[ O_2 + e^- \rightarrow O_2^- \]

Losing an electron: - oxygen of Ascorbic acid (AH$_2$) to the dehydro form (A) through a free radical intermediate (A-H).

\[ AH_2 + e^- \rightarrow AH - e^- \rightarrow A \]

This free radical can also be formed by homolytic bond fission. A covalent bond is cleaved, splits symmetrically and each of its fragment retains a single e$^-$ and become free radical. Such covalent bonds dissociate at 450-600 °C.

\[ A \times B \rightarrow A^- \times + B^- \times \]

Figure 1.1: Free radical is any atom or molecule which has an "unpaired electron" in the outer ring. An "unpaired electron" will also always mean that there is an odd number since "pairing" of electrons goes by 2s.

It is an atom with an unpaired electron in the outer ring and lacking an electron.

The reaction steps for the formation of some free radicals during the course of molecular oxygen to H$_2$O are shown here under......
Free radicals thus may be + ve charged, - ve charged or may be neutral. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen 1999). Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non radical forms and function as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Halliwell 1994; Kuhn 1976; Kumpulainen and Salonen 1999 and Younes and Siegers 1981). Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow 1990; Branen 1975). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements (Schuler 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu 2000; Koleva et. al. 2002; Mantle et. al. 2000; Oke and Hamburger 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are a
group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski et. al. 1987). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva et. al. 2002).

2.2.1. History

The first organic free radical identified was triphenylmethyl radical, by Moses Gomberg in 1900 at the University of Michigan.

Historically, the term radical has also been used for bound parts of the molecule, especially when they remain unchanged in reactions. These are now called functional groups. For example, methyl alcohol was described as consisting of a methyl "radical" and a hydroxyl "radical". Neither are radicals in the modern chemical sense, as they are permanently bound to each other, and have no unpaired, reactive electrons. They can, however, be observed as radicals in mass spectrometry when broken apart by irradiation with energetic electrons.

**Depicting radicals in chemical reactions**

In chemical equations, free radicals are frequently denoted by a dot placed immediately to the right of the atomic symbol or molecular formula as follows:

\[
\text{Cl}_2 \xrightarrow{u.v.} \text{Cl}^- + \text{Cl}^.
\]

Chlorine gas can be broken down by ultraviolet light to form atomic chlorine radicals.

Radical reaction mechanisms use single-headed arrows to depict the movement of single electrons:

\[
\text{Cl}_2 \xrightarrow{u.v.} \text{Cl}^- + \text{Cl}^.
\]

The homolytic cleavage of the breaking bond is drawn with a 'fish-hook' arrow to distinguish from the usual movement of two electrons depicted by a standard curly arrow.
It should be noted that the second electron of the breaking bond also moves to pair up with the attacking radical electron; this is not explicitly indicated in this case.

### 2.2.2. Production of Free Radicals

During normal biochemical reactions in the body, generation of reactive oxygen and nitrogen species takes place. ROMS are generated by specialized phagocytic cells (Macrophages and neutrophils) as cytotoxic agents to fight invading microbes. These phagocytes cells use membrane bound NADPH oxidase complex which catalyses one electron reduction of O$_2$ to O$_2^-$; NADPH oxidase of phagocytes transfer’s electrons from NADPH at cytosolic side of membrane of molecular O$_2$ at the other side of membrane.

\[
\text{NADPH} + 2\text{O}_2 \xrightarrow{\text{Oxidase}} \text{NADP}^+ + 2\text{O}_2^- + \text{H}^+
\]

Superoxide is converted to H$_2$O$_2$ by SOD.

\[
2\text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

The neutrophil cytoplasmic granules also contain enzyme Myeloperoxidase (MPO) (Babior 1978). The ROS are also produced by electron leakage from transport chain in mitochondria and endoplasmic reticulum where molecular O$_2$ is sequentially reduced to O$_2^-$ and H$_2$O$_2$.

### 2.2.3. Types of Free Radicals

Free radical can be of –

a) Reactive O$_2$ Species (ROS)

b) Reactive Nitrogen Species (RNS)

c) Reactive chlorine Species (RCS)

#### 2.2.3.1. Reactive Oxygen Species

Reactive oxygen species are free radicals that contain the oxygen atom. They are very small molecules that include oxygen ions and peroxides and can be either inorganic or organic. They are highly reactive due to the presence of unpaired valence shell electrons. Super oxide anions are formed when oxygen (O$_2^-$) acquires an additional electron leaving a molecule with only one unpaired electron. It is a first reduction production of O$_2$

\[
\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^-
\]
This O$_2^-$ is continuously formed in mitochondria. The rate of formation depends on the amount of O$_2$ flowing through the mitochondria. Hydroxyl radicals are short lived but they are most damaging radicals within body. This free radical can be formed from O$_2^-$ and H$_2$O$_2$ through the Harber Weiss reaction.

$$\text{O}_2^- + \text{e}^- 2\text{H}^+ \xrightarrow{\text{H}_2\text{O}} \text{OH}^-$$

The interaction of Cu or Fe and H$_2$O also produce OH$^-$ as first observed by Fenten. The oxidation potential and reactivity of various ROS is in the order (Naqui et al. 1986)

$$\text{O}_2^- < \text{H}_2\text{O}_2 < \text{O}_2 < \text{OH}^-$$

Hydrogen peroxide can be produced \textit{in vivo}. It is highly reactive and it can be converted to highly damaging hydroxyl radical or can be catalyzed and excreted harmlessly as water.
Figure 2.1: Major cellular sources of ROS in living cells.

Glutathione Peroxidase is essential for the conversion of glutathione to oxidized glutathione, during which H$_2$O$_2$ is converted to water. If H$_2$O$_2$ is converted into water O$_2$ is formed.

Singlet O$_2$ is not a free radical but can be formed during radical reactions. Singlet oxygen violates Hund’s rule of electron filling in that it has eight outer electrons existing in pairs, leaving one orbital of the same energy level empty. When the O$_2$ is energetically excited, one of the electrons can jump to empty orbital creating unpaired electrons. This singlet O$_2$ can then transfer the energy to a new molecule and act as a catalyst for a free radical formation. This molecule can also interact with other molecules leading to the formation of a new free radical.
2.2.3.2. Reactive Nitrogen Species
These include Nitric oxide, nitrogen monoxide (NO), Nitrogen dioxide (NO₂).
These are formed in the erythrocytes during oxidation of phenyl hydrazine. Both NO and NO₂ which are found in cigarette smoke, supersonic aircraft exhausts etc. contain odd no. of electrons, and therefore act as free radical.

2.2.3.3. Reactive Chlorine Species
Atomic chlorine, Cl’ Hypochlorous acid, HOCl Nitryl chloride, NO₂Cl Chloramines Chlorine gas (Cl₂) Bromine chloride (BrCl) Chlorine dioxide (ClO₂).
Chlorine replaces hydrogen on the aromatic rings of lignin via aromatic substitution, oxidizes pendant groups to carboxylic acids and adds across carbon carbon double bonds in the lignin side chains. Chlorine also attacks cellulose, but this reaction occurs predominately at pH 7, where un-ionized hypochlorous acid, HClO, is the main chlorine species in solution. To avoid excessive cellulose degradation, chlorination is carried out at pH <1.5.

\[
\text{Cl}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{Cl}^- + \text{HClO}
\]

At pH >8 the dominant species is hypochlorite, ClO-, which is also useful for lignin removal. Sodium hypochlorite can be purchased or generated in situ by reacting chlorine with sodium hydroxide.

\[
2\ \text{NaOH} + \text{Cl}_2 \rightleftharpoons \text{NaOCl} + \text{NaCl} + \text{H}_2\text{O} .
\]

2.2.4. Effect of Free Radicals
Free radicals are highly reactive and are capable of damaging of almost all types of bio molecules such as proteins, lipids, polysaccharides, nucleic acids etc. The fact is that free
radicals be get free radicals i.e. generate free radicals from normal compounds which continues as a chain reaction.

2.2.4.1. Effect on Lipids
Polyunsaturated fatty acids (PUFA) are highly susceptible to damage by free radicals. PUFA undergo peroxidation resulting in the formation of malondialdehyde (a compound that is commonly estimated in the biological fluid to assess oxidative damage). Lipid peroxidation is associated with loss of membrane function and transmembrane ionic gradient. This auto oxidation can be initiated thereby producing loss of fluidity and breakdown of the membrane’s secretary functions by the hydroxyl radical, the hydroperoxyl radical and perhaps by singlet oxygen also but not by the less reactive superoxide anion radical which removes H₂O₂. The initiating free radical removes a hydrogen atom from one of the methylene carbons of carbon chain. A process that leaves behind an unpaired electron on this carbon atom and creates a lipid carbon radical the latter rapidly undergoes molecular rearrangement to produce a conjugate dieve, which then reacts with molecular oxygen to yield a hydroperoxl radical. This radical may in turn extract a hydrogen atom from a methylene carbon of an adjacent methylene (-CH-) group of the poly unsaturated fatty acids to form another lipid radical and lipid peroxide. The lipid radical then combines with molecular oxygen and the chain reaction continues. The lipid hydroperoxide is a stable compound until it comes in contact with transition metal ions, when it produces more radicals which in turn further initiate and propagate other chain reactions. The end product of such lipid peroxidation process are aldehydes, hydro carbon gases and various chemical residues including Malondaldehyde these degradation products can diffuse away from the site of the chain reaction and give rise to cell edema and influence vascular permeability and cause inflammation and chemotaxis. These products may also alter the activity of phopholipases and induce release of arachidonic acid with subsequent formation stable prostaglandins and various endoperoxides.

2.2.4.2. Effect on Protein
Free radicals cause oxidation of sulfhydryl groups, modification of certain amino acids such as methoneine, arginin, histidine, proline. The net result is that protein loss its biological activity which may be often associated with cleavage of protein into fragments.
ROS attacks on the S-H group and HO modifies amino acid residues since LPO destroys cell membrane, it causes damage to receptor, enzyme ion channels. ROS can also modify amino acid residues of protein and lead to cross linking which results in changes in conformation and loss of function. Irreversible damage can result due to ring cleavage in tryptophan and these residues are highly susceptible to free radical damage.

2.2.4.3. Effect on Carbohydrates
Hydroxyl radicals react with sugar such as glucose, mannitol and deoxy sugar, Hyaluronic acid forms central axis to proteoglycan and maintain viscosity of synovial fluid. When exposed to free radical system, this polymer fragment leads to destabilization of connective tissue and loss of sensual fluid viscosity.

2.2.4.4. Effect on DNA
Free radicals can break DNA strands, besides alterations in nitrogen bases, which may leads to cell death or carcinogenesis. H$_2$O$_2$ is known to cause DNA breaks in intact cells and purified DNA. MDA (Malondialdehyde) is the major end product and an index of LPO, cross links DNA and proteins and nucleotides on same and opposite and readily reacts with deoxynucleosides to produce adducts. OH also formation of many type of oxidized nucleosides 8- HDG (Jones et. al. 1981). The damage is not repaired mutation also accumulate, with the leading to carcinogenesis and degenerative disease.

2.3. ANTIMICROBIAL ACTIVITIES OF MEDICINAL PLANTS
Diseases caused by the bacteria are widespread worldwide. The treatment of these infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through genes (Davis 1994). In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions (Ahmad et. al. 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious disease from various sources of medicinal plants.

Undoubtedly, medicinal plants are the prime source of drugs in both developing and developed nations, as drugs or herbal extracts for various chemotherapeutic purposes. There are about 2000 plants species known to possess medicinal value in traditional Asian system of medicine (Agnese et. al. 2001). The use of derive natural compounds
used as alternative sources of medicine continues to play major roles in general wellness of people all over the world. The curative properties of medicinal plants are due to the presence of complex chemical substances of different compositions which occur as secondary metabolites (Karthikeyan et. al. 2009). They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids and essential oils. Over 50% of all modern clinical drugs are of natural origin and play an important role in development of drugs. Many herbs have been used for treating disease caused by microorganism such as cholera, diarrhea, dysentery, typhoid and bacterial enteritis. Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative antimicrobial regimens.

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, latin America and Africa is reported to have minimal side effects (Bibitha et. al. 2002; Maghrani et. al. 2005).

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need of newer antibiotic sources.

Because of its wide usage and availability, various studies were set out to investigate the antimicrobial activity of plants.

2.4. ANTIOXIDANT

Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, liver disease, macular degeneration, diabetes, cancer etc are all contributed by oxidative damage. Antioxidants may also enhance immune defense and therefore lower the risk of cancer and infection.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants,
such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Vertuani et al. 2004).

2.4.1. Exogenous Antioxidants
- Natural synthetic product which is introduced into body from outside.

Example of antioxidants
Most popular antioxidant food additives have been BHA (butylated hydroxy anisole), BHT (butylated hydroxytoluene) propyl gallate and tocopherol vitamin E, beta carotene, vitamin C, Additionally selenium a trace metal that is required for proper function of one of the body’s antioxidant enzyme systems. The body cannot manufacture these micro nutrients so they must supplied in the diet.
Antioxidants may also use commercial purpose:
(1) Such as low density polythene is protected by antioxidants like Carbon black which absorbs the ultra violet light which causes free radical production.
(2) Some oils also have small quantities of antioxidants such as phenol and amine derivatives.
(3) Plastics are often formed by free radical action; they can also be broken down by the same process.
(4) Some antioxidants are added into paints so that vital lubricating oils should remain stable and liquid should not dry.
(5) The irradiation of food, which is an excellent way of killing bacteria that can cause spoilage and can be dangerous. It also causes the free radical formation that can lead to
unacceptable chemical changes in food, so it is also necessary to counteract the undesirable effects of irradiation of food by the use of antioxidants. There are also some natural body antioxidants which include compounds such as cystine glutathione and D- penicillamine and blood constituents such as the iron containing molecule transferrin and the protein ceruloplasmein. These act either by preventing free radicals from being produced or by mapping them up.

The body also contains a no. of important antioxidant enzymes. An enzyme is a highly active protein that accelerates the chemical reaction.

2.4.2. **Endogenous Antioxidants**

They are present in biological system under physiological conditions. They provide important defense mechanism that allows aerobic organisms to cope with daily challenges of oxidative stress.

There are two types of defense systems against these free radicals

- Enzymatic
- Non enzymatic

2.4.2.1. **Enzymatic**

Cells contain several enzyme systems for removing oxygen derived radicals and their product. They can act by quenching of O$_2^-$, decomposition of H$_2$O$_2$ and sequestration of metal ions. These include enzymatic SOD, catalase glutathione peroxides, glutathione reductase and non enzymatic like minerals and some proteins. Such protective antioxidants are:

- Superoxide dismutase
- Catalase $\rightarrow$ H$_2$O$_2$ (Oxidoreductase)

2.4.2.1.1. **Superoxide Dismutase**

They are the family of metallo-enzymes that convert O$_2^-$ to H$_2$O$_2$.

$$O_2^- + O_2^- \xrightarrow{2hr/SOD} H_2O_2 + O_2$$

It is the most important enzyme because it is found virtually in all aerobic organisms for *E.coli*. These SOD enzymes have been purified one of which is Mn SOD being and iron containing enzyme (Fe SOD). Human SOD is Cu-Zn-SOD enzyme. The transition metal of an enzyme reacts with O$_2^-$ taking its O$_2$ the only known substrate for SOD, Cu-Zn-
SOD is found in cytosol of most of the Eukaryotic cells. Mn-SOD is located in the mitochondrial matrix and bacteria. Mn-SOD has been identified as a mediator of survival which is expressed at higher levels. SOD is considered to be a stress protein, which is synthesized in response to oxidative stress.

The structure of iron SOD from *E.coli* has been determined at 31ºA resolution and from *Pseudomonas ovalis* at 2.9ºA resolution. Iron containing SOD usually contains two subunits. The dimeric enzyme usually contains one or two ions of iron per molecule of the enzyme. OH or Fe (II) O generated from the metal catalysed interaction of O₂ with H₂O₂, the in vitro process is inhibited by SOD or Catalase or by chelating agents. Cu-Zn-SOD inhibition reduces Cu II at active site but more slowly it activates the reduced enzyme, which is prevented by Xanthine, urate and formate (Fridovich 1976).

SOD can act as anticarcinogenic and inhibitor at initiation and promotion/transformation stage in carcinogenesis. SOD production increased in response to ROMS produced in various genetic diseases.

2.4.2.1.2. Catalase

It is one of the most thoroughly investigated mammalia enzyme. It catalyzes the decomposition of hydrogen peroxide to water with the liberation of oxygen.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

It is present in most cells. CAT is a heme protein. It is $10^4$ times faster then peroxides. It was first crystallized from beef liver (Handler and Thurman 1990). It has since been crystallized from liver and erythrocytes of a number of animal species including humans. It is localized mainly in mitochondria and sub cellular respiratory organelle, 80% in peroxisomes and 20% cytosol (mol.wt. →2,40,000) 4 subunits of equal size each containing a heme (iii) fe protoporphyrin group bound to its active site (Beers and Seizer 1952). Catalase is able to catalyze the decomposition of H₂O₂ by two separate types of reactions depending upon the condition. Both reactions begin with the formation of primary complex between H₂O₂ and iron of the heme prosthetic group.

\[
\text{Enz-Fe}^{3+} - \text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{ENZ-Fe}^{3+} - \text{OOH} + \text{H}_2\text{O}
\]

This is called primary complex and it reacts with a second molecule of hydrogen peroxide in the catalytic destruction of hydrogen peroxide as follows:-

\[
\text{Enz-Fe}^{3+} - \text{OOH} + \text{H}_2\text{O}_2 \rightarrow \text{Enz-Fe}^{3+} - \text{OH} + \text{H}_2\text{O} + \text{O}_2
\]
Catalase, a marker enzyme of peroxisomes, reacts either catalytically or peroxodetically depending on the microenvironment of the cell. It plays a protective role against oxygen toxicity by degradation of H$_2$O$_2$ produced in several metabolic reactions.

**Distribution:** Catalase is widely distributed in various organisms, from bacterial to plants and protozoa to mammals. It is most abundant in hepatic cells, kidney and erythrocytes. A number of records indicated that the activity associated with the cytosole and with the large no of granule fractions, particularly peroxisomes (Holmes and Masters 1970; Chance 1949).

It functions as promotion /transformation inhibitor in carcinogenesis. It is found to reduce SCE levels resulting from treatment with H$_2$O$_2$. CAT also prevented chromosomal aberrations caused by hypoxanthine or in Chinese hamster cells. CAT activity was found to be lower in tumor (breast cancer) tissue than in normal tissue.

### 2.4.2.2. Non Enzymatic

#### 2.4.2.2.1. Vitamin E

**Vitamin E:** d-alpha tocopherol is a fat soluble vitamin, discovered in 1922. It was found that female rats required an unknown substance in their diet for the normal pregnancies. It is not present in diet, they could ovulate and conceive satisfactorily but with in 10 days the foetus invariably died. In case of male rats it was observed that abnormalities in their testes (Burtan 1988). So it is also called as an anti-sterility vitamin, also used in treatment of various menstrual disorders and infertility. Majority of it is found in nuts, seeds, vegetable and fish oils, whole grains (esp. wheat germ) fortified cereals and apricots.

Current recommended daily allowance (RDA) is – 15 IU/day for man and 12 IU/day for women. The IU is equal to 1mg of alpha tocopherol acetate. It is almost insoluble in water but dissolves in oils, fats, alcohol. Daily uptake = 10-30 mg

**Deficiency causes:** Changes in brain and nervous system walking disturbance, anemia, Odema, skin disorders.

**Over dosage:** Danger for premature babies.

#### 2.4.2.2.2. Vitamin C

**Vitamin C:** Ascorbic acid is a water-soluble vitamin. It was isolated in 1928 and chemically identified in 1932 (Burtan 1988). It is readily destroyed by exposure to air by
cooking especially in the presence of copper and alkalies. Vitamin C is necessary for the proper synthesis of collagen which is the main constituent of the bones and other tissues. Daily intake should → 60mg a day

People need more while suffering from infectious diseases, injuries, burns, rheumatic disorder and after surgical operations. Ascorbic acid is found in cabbage, spinach, broccoli, kale, cantaloupe, kiwi, strawberries and citrus fruits.

**Deficiency causes:** Scurvy, failure of wounds to heal, looseness of teeth in babies and small children. Scurvy also causes bleeding under the bone membranes, causing very tender swelling in infants.

**2.4.2.2.3. β -Carotene**

It is a precursor of vitamin A (Retinol). It is also called as pro vitamin A because it is converted into vitamin A (retinal and other forms) in the liver. They are necessary for the growth and health of the surfaces and lining tissues and the bones, for the immune system and for protection against cancer for normal vision and for corneas protection against skin diseases, sunlight radiation found in liver, egg yolk, milk, butter, yellow and green vegetables, fish, spinach, carrots, squash, broccoli, yams, tomato, peaches and grains. Pregnant lady should take 7-12 mg a day during first three months of pregnancy.

**Deficiency causes:** Night blindness, dryness of eye xerophthalmia. Babies may suffer devastating melting of the corneas of eyes with permanent blindness.

**Over dosage:** Over dosage causes chronic poisoning with skin dryness, itching peeling, drowsiness, irritability, loss of appetite, tender swelling over the bones and enlargement of liver and spleen.

**2.5. BIOMEDICAL IMPORTANCE OF ANTIOXIDANTS**

**2.5.1. Carcinogenesis**

A no. of different free radical generating compound enhance malignant conversion of appaloosas into carcinoma and their effectiveness may be related to type of radicals produced in biological system. Significant level of DNA damage occurs by oxy radicals leading to mutation, deletion, gene amplification or rearrangements and is responsible for various etiologies of human cancer.

**2.5.2. Inflammatory Diseases**
Intracellular protection of cytoplasmic components against phagocyte derived oxidative injury is mediated predominantly by antioxidant enzymes like SOD, catalase and glutathione peroxidase. Mutation in p53 gene and other regulatory genes promotes inflammation into chronic disease, in rheumatoid arthritis and other inflammatory disorders.

2.5.3. Respiratory Disease
Oxidation stress plays an important role in pathogenesis of number of long diseases like chronic obstructive pulmonary diseases (COPD), bronchial asthma and acute respiratory disease syndrome. Important consequence of it is COPD including oxidation inactivation of anti-protease air space epithelial injury, increased sequestration of neutrophils in pulmonary micro vasculature and gene expression of pro-inflammatory mediators.

2.5.4. Infectious Disease
H.I.V and opportunistic infections directly or indirectly lead to an oxidative stress, caused by excess generation of ROS/RNS by activated cells of immune system. In early stages of H.I.V infection, plasma / lymphocyte SOD level is decreased.

2.5.5. Ageing
Increased accumulation of free radicals causes apoptosis, necrosis and cell death and thus is responsible for various changes in physical characteristics and decline in many physiological functions.

2.6. METABOLISM OF ALCOHOL
Alcohol (Ethanol): Ethanol, the only form of alcohol, is absorbed mainly in the intestine, where it is channeled through the portal vein directly toward the liver before passing through the circulatory system and the rest of the body. Three enzymatic systems are able to carry out the ethanol oxidation:

1. Alcohol dehydrogenase (ADH)
2. The microsomal ethanol-oxidizing system (MEOS)
3. Catalase (CAT) (Riveros-Rosas et al. 1997)

Ethanol is metabolized mostly by ADH, an enzyme which couples the oxidation of ethanol into acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD\(^+\)).

\[
\text{Ethanol} \xrightarrow{\text{ADH}} \text{Acetaldehyde} \quad \text{reduction} \quad \text{NAD}^+
\]

MEOS system connects ethanol and nicotinamide adenine dinucleotide phosphate (NADPH) oxidation to the reduction of molecular oxygen to hydrogen peroxide, and requires the participation of the P-450 cytochrome (Lieber 2005).

In the third system, the oxidation of an ethanol molecule into acetaldehyde is linked to the simultaneous decomposition of a hydrogen peroxide in a reaction catalyzed by the CAT enzyme (Handler and Thurman 1990).

Chronic ethanol ingestion leads to the formation of reactive oxygen species (ROS), (Mantel and Preedy 1999) and can induce a decrease of antioxidant defense (Oh et al. 1998). Thus, chronic and excessive alcohol consumption may accelerate oxidative mechanism directly or indirectly, which eventually produces cell death and tissue damage (Sun et al. 2001). Experimental studies on animal models have also indicated that ethanol enhanced the fatty acid oxidation by kidney microsomes and peroxisomes, (Orellana et al. 1998) and affected the activities of some kidney lysosomal hydrolases (Witek and Kolataj 1999). Oxidative stress and ROS-mediated toxicity have been considered the primary routes to alcohol-induced kidney injury (Orellana et al. 1998; Scott et al. 2000; Rodrigo et al. 2002). Oxidative stress is the term used to describe an imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage (Sies 1991).
Chronic alcohol intake leads to hepatic injury, including apoptotic cell death, representing a major health treat in alcoholics (Zhou et. al. 2001). Although alcohol consumption in the US is declining, over 14 million people still meet the diagnostic
Nutraceutical evaluation of edible plants Colocasia esculenta and Girardinia heterophylla from high altitude

criteria for alcoholism, of which 2 million suffer from alcoholic liver disease, with 14000 deaths each year as a result of alcoholic liver cirrhosis (Seitz et. al. 2005). The liver serves as the primary site of oxidation where ethanol is metabolized to various metabolites (Zhou et. al. 2001). Acetaldehyde, the first oxidized metabolic product of ethanol, has been considered a candidate toxin for alcohol-induced organ damage, possibly through direct cytotoxicity and the release of inflammatory cytokines (Zhou et. al. 2001; Cederbaum 2001; Lieber 2003).

2.7. LIVER

2.7.1. Anatomy

An adult human liver normally weighs between 1.4–1.6 kg (3.1–3.5 lb), (Cotran et. al. 2005) and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder.

The liver receives a dual blood supply from the hepatic portal vein and hepatic arteries. Supplying approximately 75% of the liver's blood supply, the hepatic portal vein carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs. The hepatic arteries supply arterial blood to the liver, accounting for the remainder of its blood flow. Oxygen is provided from both sources; approximately half of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic-arteries. Blood flows through the sinusoids and empties into the central vein of each lobule. The central veins coalesce into hepatic veins, which leave the liver and empty into the inferior vena cava.
The term biliary tree is derived from the arboreal branches of the bile ducts. The bile produced in the liver is collected in bile canaliculi, which merge to form bile ducts. Within the liver, these ducts are called intrahepatic (within the liver) bile ducts, and once they exit the liver they are considered extrahepatic (outside the liver). The intrahepatic ducts eventually drain into the right and left hepatic ducts, which merge to form the common hepatic duct. The cystic duct from the gallbladder joins with the common hepatic duct to form the common bile duct.

Bile can either drain directly into the duodenum via the common bile duct or be temporarily stored in the gallbladder via the cystic duct. The common bile duct and the pancreatic duct enter the second part of the duodenum together at the ampulla of Vater.
Apart from a patch where it connects to the diaphragm (the so-called "bare area"), the liver is covered entirely by visceral peritoneum, a thin, double-layered membrane that reduces friction against other organs. The peritoneum folds back on itself to form the falciform ligament and the right and left triangular ligaments. These "ligts" are in no way related to the true anatomic ligaments in joints, and have essentially no functional importance, but they are easily recognizable surface landmarks. An exception to this is the falciform ligament, which attaches the liver to the posterior portion of the anterior body wall.

Traditional gross anatomy divided the liver into four lobes based on surface features. The falciform ligament is visible on the front (anterior side) of the liver. This divides the liver into a left anatomical lobe, and a right anatomical lobe. If the liver flipped over, to look at it from behind (the visceral surface), there are two additional lobes between the right and left. These are the caudate lobe (the more superior), and below this the quadrate lobe. From behind, the lobes are divided up by the ligamentum venosum and ligamentum teres (anything left of these is the left lobe), the transverse fissure (or porta hepatics) divides the caudate from the quadrate lobe, and the right
sagittal fossa, which the inferior vena cava runs over, separates these two lobes from the right lobe. Each of the lobes is made up of lobules; a vein goes from the centre of each lobule which then joins to the hepatic vein to carry blood out from the liver. On the surface of the lobules there are ducts, veins and arteries that carry fluids to and from them.

2.7.2. Biochemical Function

The liver regulates most chemical levels in the blood and excretes a product called bile, which helps carry away waste products from the liver. All the blood leaving the stomach and intestines passes through the liver. The liver processes this blood and breaks down the nutrients and drugs into forms that are easier to use for the rest of the body. More than 500 vital functions have been identified with the liver. Some of the more well-known functions include the following:

- production of bile, which helps carry away waste and break down fats in the small intestine during digestion.
- production of certain proteins for blood plasma.
- production of cholesterol and special proteins to help carry fats through the body.
- conversion of excess glucose into glycogen for storage (glycogen can later be converted back to glucose for energy)
• regulation of blood levels of amino acids, which form the building blocks of proteins.
• processing of hemoglobin for use of its iron content (the liver stores iron)
• conversion of poisonous ammonia to urea (urea is an end product of protein metabolism and is excreted in the urine)
• clearing the blood of drugs and other poisonous substances.
• regulating blood clotting.
• resisting infections by producing immune factors and removing bacteria from the bloodstream.

When the liver has broken down harmful substances, its by-products are excreted into the bile or blood. Bile by-products enter the intestine and ultimately leave the body in the form of faeces. Blood by-products are filtered out by the kidneys, and leave the body in the form of urine.

2.7.3. Effect of Alcohol on Liver
Alcoholic liver disease is the major cause of liver disease in Western countries, (in Asian countries, viral hepatitis is the major cause). It arises from the excessive ingestion of alcohol. Even though millions of individuals drink alcohol on a regular basis, only a few heavy drinkers develop liver damage. The liver damage induced by alcohol is not sudden as it occurs slowly over a period of 10-15 years (Friedman et. al. 2003). How alcohol damages the liver is not completely understood. It is known that alcohol produces toxic chemicals like acetaldehyde which can damage liver cells, but why this occurs in only a few individuals is still in debate. When alcohol damages the liver, the function of the organ is not immediately compromised as the liver has a tremendous capacity to regenerate and even when 75% of the liver is damaged, it continues to function as normal. Thus when alcohol-induced liver damage produces symptoms, there is always significant damage which by now is usually irreversible. When alcohol is consumed for a long time, it eventually results in liver scarring or what is known as cirrhosis or end-stage alcoholic liver disease (McNally and Peter 2006).
Types of alcohol-induced liver damage

Alcohol-related liver damage can be divided into three categories (French et. al. 1993):

• **Fatty liver.** Some degree of fat deposition in the liver occurs in almost all heavy drinkers. It also may occur transiently in nonalcoholics after a single drinking session. Fatty liver is reversible and is not believed to lead to more serious damage.

• **Alcoholic hepatitis.** This disorder is characterized by widespread inflammation and destruction (i.e., necrosis) of liver tissue. Scar tissue may begin to replace healthy liver tissue, a process called fibrosis. Symptoms of alcoholic hepatitis may include fever, jaundice, 2 and abdominal pain.

• **Alcoholic cirrhosis.** This most advanced form of liver disease is diagnosed in 15 to 30 percent of heavy drinkers. Between 40 and 90 percent of the 26,000 annual deaths from cirrhosis are alcohol related (Dufour et. al. 1993). A cirrhotic liver is characterized by extensive fibrosis that stiffens blood vessels and distorts the internal structure of the liver. This structural damage results in severe functional impairment, which may lead
secondarily to malfunction of other organs, such as the brain and kidneys. Although alcoholic cirrhosis is usually fatal because of complications (e.g., kidney failure and hypertension in the vein carrying blood to the liver [i.e., the portal vein]), it can stabilize with abstinence. Traditionally, these three conditions have been considered sequentially related, progressing from fatty liver to alcoholic hepatitis to cirrhosis. However, heavy drinkers may develop alcoholic cirrhosis without first developing hepatitis. Moreover, alcoholic hepatitis may have a sudden onset and a rapid course, causing death before cirrhosis can develop.

Possible mechanisms influencing alcohol-induced liver damage

The mechanisms that influence liver injury are both poorly understood and controversial. Moreover, they interact in complex ways. The following sections briefly discuss aspects of these mechanisms and their interactions.

Role of Oxygen Oxygen-related factors that influence alcohol-induced liver damage include the effects of free radicals, antioxidants, and hypoxia.

Free Radicals Much of the direct cell damage that occurs during alcoholic liver disease is believed to be caused by free radicals. Free radicals are highly reactive molecular fragments that frequently contain oxygen. Small quantities of free radicals are produced as normal by-products of various metabolic processes. These fragments are quickly scavenged by natural protective molecules in the cell, called antioxidants (e.g., glutathione and vitamins A and E). However, when free radicals are produced in excess or when antioxidant defenses are impaired, the free radicals may interact destructively with vital cell constituents, potentially causing death of the cell.

One common result of free radical attack is the sequential degradation of cell membranes by a process known as lipid peroxidation. This process may destroy the integrity of the membranes both within and surrounding the cell, seriously compromising cell function (Rubin 1993). Researchers have demonstrated that chronic alcohol consumption induces lipid peroxidation in rats and that the degree of lipid peroxidation is related to the extent of liver injury (Nanji et al. 1994). When alcohol is metabolized in liver cells by CYP2E1, free radicals are produced. In rats, which are somewhat resistant to alcoholic
liver injury, disease can be induced when alcohol is administered together with a high-fat diet (Tsukamoto et al. 1990). The fat increases CYP2E1 activity and simultaneously alters membrane composition, making the membrane more susceptible to peroxidation (Nanji et al. 1994).

**Antioxidants** As mentioned previously, antioxidants are the cell’s defense against free radicals. Chronic alcohol consumption diminishes the levels of these antioxidants and renders liver cells more susceptible to free radical-induced injury. One important antioxidant that is affected by alcohol is glutathione. Liver cells contain an abundance of glutathione, especially within structures called mitochondria, where most of each cell’s energy is generated. The key enzymes in mitochondria are certain cytochromes that are integral components of the inner mitochondrial membrane. Like CYP2E1, these cytochromes can produce free radicals—hence the need for antioxidant protection. Glutathione is not synthesized in mitochondria; adequate concentrations of glutathione are maintained there by active transport from the cytoplasm through the mitochondrial membrane. Alcohol interferes with the transport of glutathione through membranes, leading to its depletion from mitochondria. The resulting glutathione deficiency may permit mitochondrial damage and cell death by means of unimpeded lipid peroxidation. Similarly, alcohol has been found to decrease concentrations of vitamins A and E in rat livers, resulting in increased lipid peroxidation and liver injury.

**Hypoxia (Low Oxygen Concentration)** Alcohol metabolism appears to increase oxygen utilization by liver cells, thereby reducing the availability of oxygen for other important cellular functions. This phenomenon is most important in zone 3 of the liver lobules, which normally is exposed to lower concentrations of oxygen than zone 1 or zone 2. The tendency of hypoxia to occur in zone 3, together with the fact that free radicals are more likely to be formed in this region, may account for the observation that alcoholic liver damage tends to concentrate in zone 3. Cells lining the liver sinusoids also may contribute to hypoxia by secreting endothelin, a potent agent that induces narrowing of blood vessels. The resulting narrowing of the sinusoids may decrease the delivery of oxygen-containing blood to zone 3. Patients with cirrhosis also experience increased levels of plasma endothelin, compared with healthy subjects.
**Inflammatory Agents** Inflammation is a localized defensive response to tissue injury. Liver inflammation is the hallmark of alcoholic hepatitis. The inflammatory process begins when liver cells release chemicals that attract specialized white blood cells, or phagocytes, to the damaged tissue. Phagocytes engulf and destroy foreign substances, detoxify bacterial poisons, produce antibodies, and release chemical messengers that attract more phagocytes to the area. Phagocytes arriving from the bloodstream are assisted by a population of phagocytes that remain permanently in the liver, known as Kupffer cells. These activities form a highly complex, interrelated, exquisitely regulated network for protection against disease-causing microorganisms and cancer. Under certain circumstances, such as heavy alcohol consumption, the inflammatory process can threaten the body’s own tissues. For example, chronic heavy alcohol consumption can cause an imbalance of certain biological molecules and set in motion other mechanisms leading to tissue damage. Three classes of molecules (i.e., eicosanoids, cytokines, and endotoxins) and two processes (i.e., adduct formation and fibrosis) are the subjects of the following sections.

**Eicosanoids** Eicosanoids are a family of biological molecules with a wide range of functions. Different eicosanoids affect the liver in different ways: Prostaglandins and prostacyclins can protect liver cells from certain kinds of damage; conversely, thromboxanes cause blood vessels to narrow, which can promote hypoxia or directly cause inflammation or necrosis (Nanji *et. al.* 1993). Another type of eicosanoid, the leukotrienes (e.g., leukotriene B4), may cause liver injury by attracting and activating neutrophils, special white blood cells with phagocytic properties. Long-term alcohol consumption alters the balance of eicosanoids in the liver by decreasing the production of cell-protective prostaglandins and prostacyclins and by increasing the synthesis of the harmful eicosanoid thromboxane B2 and, possibly, leukotriene B4 (Nanji *et. al.* 1993).

**Cytokines** Cytokines are a family of chemicals produced by various immune system cells, including the Kupffer cells of the liver. As with eicosanoids, the cytokines have many overlapping biological functions, and they can be harmful in the context of long-term heavy alcohol consumption (McClain *et. al.* 1993). Patients with alcoholic hepatitis frequently have high levels of cytokines in their bloodstream, including tumor necrosis
factor alpha (TNF-α) (McClain et. al. 1993). TNF-α, produced primarily by Kupffer cells, may cause liver injury directly or indirectly. First, evidence suggests that TNF-α might be directly toxic to liver cells.

Second, TNF-α stimulates the liver to produce other cytokines, which attract white blood cells to the liver and stimulate them to release free radicals and toxic enzymes. In experimental animals, TNF-α increases in the liver after 1 month of alcohol administration, timing that coincides with the onset of liver cell necrosis and inflammation. The mechanisms of increased cytokine production in alcoholic liver disease are not well understood. Chronic exposure to bacterial toxins in the alcoholic may stimulate inflammatory cytokine production (McClain et. al. 1993).

**Endotoxins** Endotoxins are major molecular constituents of the outer membrane of certain bacteria. Chemically, endotoxins are complex molecules called lipopolysaccharides (LPS’s). These chemicals cause many of the toxic effects of bacterial infection on most organ systems. Endotoxins are present in the intestine because bacteria typically reside there; under normal circumstances, minute amounts of endotoxin may pass through the intestinal lining into the bloodstream. However, alcohol ingestion is believed to increase intestinal permeability to endotoxins, permitting their access to the circulation and subsequently to the liver. Endotoxins are often detectable in the blood of patients with liver disease. Upon reaching the liver, endotoxins are presumed to act on Kupffer cells, stimulating them to release chemicals that promote inflammation and hypoxia. The ongoing alcohol induced LPS absorption may provide a continuing stimulus to Kupffer cells that perpetuates inflammation in alcoholic liver disease.

**Adduct Formation** Highly reactive compounds, such as acetaldehyde and some free radicals, can attach chemically to proteins in the blood and liver. The resulting hybrid molecules are called adducts. The body’s immune system may perceive these proteins adducts as foreign and attack them with cellular toxins, white blood cells, and antibodies (Israel et. al. 1988). Antibodies directed against protein adducts have been detected in the blood of alcoholics. Adduct formation may contribute to alcoholic liver injury, either by impairing the function of the affected protein or by stimulating an immune mechanism that in turn attacks healthy liver cells.
Fibrosis Fibrosis is a major mechanism of liver disease because it can lead to irreversible cirrhosis. Longterm alcohol consumption stimulates the liver’s fat-storing (i.e., stellate) cells to produce collagen, the protein that forms scar tissue. The precise stimulus that initiates this process is unknown. Research using liver cells grown in culture indicates that compounds associated with alcoholic liver injury may be involved. For example, acetaldehyde and aldehyde-protein adducts can increase collagen synthesis in vitro, as can the chemical products of lipid peroxidation. A third potential stimulus to alcoholic fibrosis is a cytokine called transforming growth factor beta (TGF-Beta). In the presence of this cytokine, stellate cells grown in culture begin to synthesize collagen. In rats, chronic alcohol feeding induces TGF- Beta production by Kupffer cells. TGF- Beta also is produced by stellate cells themselves; this production can occur in the liver of alcoholics, creating a self perpetuating cycle of fibrosis. Kupffer cells may produce compounds other than TGF- Beta that influence the development of alcoholic liver fibrosis. For example, Matsuoka and colleagues (1990) have shown that Kupffer cells from alcohol-fed rats secrete a growth factor for stellate cells. By increasing the number of stellate cells, Kupffer cells can increase fibrosis indirectly.

Treatment of liver disease

Alcoholic Hepatitis Potential treatments for alcoholic hepatitis are largely directed against inflammation and free radical-induced liver injury.

Corticosteroids Among other functions, corticosteroids act to suppress inflammation. Natural corticosteroids are synthesized from cholesterol by the adrenal glands, which are located above the kidneys. Synthetic corticosteroids, such as prednisolone, are widely used medicinally to suppress inflammation (McClain et. al. 1993). Other studies have reported significantly improved survival rates only in patients suffering brain-related complications of alcoholic liver disease, however, and not in patients with milder illness (Imperiale and McCullough 1990).

Nutrition/Antioxidants Although it has not been shown to directly improve patient survival, aggressive nutritional support is recommended for all patients with alcoholic liver disease. Given the importance of free radicals as a cause of liver injury,
supplementation with antioxidants is a key nutritional goal. Glutathione depletion has been prevented in alcohol-fed animals by administering S-adenosyl-L-methionine (SAM), a precursor of glutathione. Interestingly, SAM’s positive effect seems unrelated to its potential promotion of glutathione synthesis, but apparently derives from its ability to modify the mitochondrial membrane, thereby restoring normal transport of glutathione through that membrane. This effect helps to maintain normal levels of glutathione in the mitochondrion, where it is needed to prevent free radical damage. Researchers also are investigating vitamins A and E as therapeutic agents for alcoholic liver disease. So far, vitamin E supplements have not significantly prevented or reversed alcoholic liver injury in experiments with laboratory animals. Vitamin A supplementation is not practical because the inherent toxicity of vitamin A severely limits the dose that can be safely administered (Lieber 1994).

**Antibiotics and Immune System Inhibitors** The role of LPS’s in alcoholic liver disease suggests that using antibiotics to eradicate LPS-containing bacteria from the gut may be helpful. For example, Adachi and colleagues (1995) demonstrated in rats that intestinal sterilization could prevent alcohol-induced liver injury. Along similar lines, researchers have suggested using antibodies or other targeted agents to block the actions of Kupffer cells or cytokines. Antibodies against LPS may be of some use (McClain et. al. 1993). In addition, thromboxane inhibitors have prevented necrosis and inflammation in alcohol-treated rats.

**Alcoholic Cirrhosis** Up to a point, liver fibrosis may be reversible with abstinence. Eventually, however, the liver loses its ability to reabsorb scar tissue, and the disease progresses to cirrhosis. Treatment for cirrhosis is directed largely against its symptoms (e.g., bleeding in the esophagus) and complications (e.g., portal vein hypertension).

**Liver Transplantation** For patients becoming terminally ill, liver transplantation is the only effective treatment. In alcoholic cirrhotic patients, liver transplantation has demonstrated both success and survival rates equal to those for nonalcoholic subjects (Kumar et. al. 1990; Van Thiel et. al. 1991).
Polyunsaturated Lecithin Lieber and colleagues (1994) demonstrated that a mixture of fatty substances called polyunsaturated lecithin (PUL) dramatically reduced the incidence of cirrhosis in baboons fed alcohol for several years. In some cases, removal of PUL from the diet led to the development of cirrhosis, perhaps in an accelerated manner (Schenker and Halff 1993). PUL presumably exerts its beneficial effect by promoting the degradation of collagen, thereby inhibiting fibrosis. PUL also may help stabilize membranes and encourage the synthesis of cell-protective prostaglandins.

2.7.4. Mode of Mechanism of Alcohol Toxicity

An understanding of alcohol metabolism provides the basis for understanding alcohol-induced liver damage. Most of the alcohol that people drink is metabolized in the liver. The major pathway for alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH). This enzyme converts alcohol to acetaldehyde through a chemical process called oxidation. Acetaldehyde is highly toxic to the body, even in low concentrations. Normally, however, the enzyme aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate. Most of the acetate travels through the bloodstream to other parts of the body, where it can enter other metabolic cycles (Lieber 1994) that produce energy or useful molecules. The usual biological role of both ADH and ALDH is to metabolize vitamin A (i.e., retinol). The microsomal enzyme oxidizing system (MEOS) is an
alternate pathway for alcohol metabolism in the liver. Microsomal enzymes belong to a family of proteins called cytochromes. Some cytochromes, located in a cellular substructure called the endoplasmic reticulum, detoxify harmful substances that enter the body. The MEOS oxidizes alcohol to acetaldehyde by means of a cytochrome called P450 2E1, or CYP2E1, which is found in the endoplasmic reticulum of liver cells. Normally functioning at a low level, CYP2E1 is stimulated (i.e., induced) to a higher level by the presence of alcohol. Thus, the MEOS becomes increasingly important as alcohol consumption becomes heavier and more chronic.

Cirrhosis is a buildup of scar tissue that changes the structure of the liver and blocks blood flow. Cirrhosis can be caused by alcoholic hepatitis, which is, of course, caused by overdrinking. Cirrhosis can cause varicose veins, which can rupture and potentially triggering internal bleeding.

The ADH Pathway The ADH pathway, which converts alcohol to the toxic substance acetaldehyde in a reaction that releases hydrogen atoms, is responsible for most of the alcohol breakdown in liver cells. However, how fast alcohol is broken down by this pathway depends, at least in part, on nutritional factors. For example, low-protein diets reduce the levels of ADH in the liver, lowering the rate of alcohol breakdown both in humans and in laboratory animals. Prolonged fasting also has been shown to decrease the rate of alcohol breakdown in isolated rat liver cells. These observations suggest that for any given alcohol dose, malnourished alcoholics break down the alcohol more slowly and therefore develop higher blood alcohol levels, and sustain them longer, than well-nourished subjects. Because the effects of alcohol on the body depend on blood alcohol levels, reduced alcohol degradation may lead to more severe damage to the liver and other organs.

Conversely, alcohol metabolism by the ADH pathway also may influence metabolic functions. As mentioned above, ADH-mediated breakdown of alcohol generates hydrogen atoms in addition to acetaldehyde. These hydrogen atoms interact with a molecule called nicotinamide adenine dinucleotide, converting it to reduced NAD. NADH, in turn, participates in many essential biochemical reactions in the cell, and in the
process passes on its hydrogen to other molecules. For proper functioning of the cell, the ratio of NAD to NADH must be tightly controlled. When alcohol metabolism generates excess amounts of NADH, the cell can no longer maintain the normal NAD/NADH ratio. This altered NAD/NADH ratio may lead to several metabolic disorders. For example, elevated levels of NADH cause the formation of abnormally high levels of lactic acid, which in turn reduce the capacity of the kidney to excrete uric acid. Excessive uric acid in the body can exacerbate gout, a disorder characterized by extremely painful swelling of certain joints. Therefore, alcohol-induced increases in NADH levels and, subsequently, uric acid levels, which can be worsened by other alcohol-induced metabolic effects, may at least partly explain the common clinical observation that excessive alcohol consumption causes or aggravates attacks of gout.

In addition, increased NADH promotes the generation of the building blocks of fat molecules and reduces the breakdown of fats in the liver, thereby contributing to fat accumulation in that organ. Other alcohol-related mechanisms also contribute to fat accumulation in the liver, including: decreased excretion of fat-containing proteins from the liver. Release of fats from other tissues, which then are transported to the liver. Enhancement of the liver's uptake of fats circulating in the blood. The resulting fatty liver is the earliest stage and the most common form of alcohol-induced liver disease. In addition to contributing to the development of fatty liver, the increases in NADH levels resulting from the ADH-mediated breakdown of alcohol also may play a role in the formation of scar tissue that characterizes fibrosis, a more severe stage of liver disease. This relationship was suggested by the observation that a molecule that can capture hydrogen away from NADH completely prevents certain liver cells (stellate cells) from producing elevated levels of molecules that contribute to the formation of scar tissue.

**The Microsomal Ethanol-Oxidizing System (MEOS)** After moderate alcohol consumption, most of the ingested alcohol is broken down by the ADH pathway described above. After chronic heavy alcohol consumption, the MEOS pathway of alcohol metabolism becomes more important. This pathway consists of several enzymes located in the liver microsomes - small spherical structures found in all cells. The MEOS has been investigated extensively because its activity increases substantially after long-term alcohol consumption and because it is important for the breakdown and elimination
of other foreign molecules from the body, including certain medications. Therefore, activation of the MEOS after alcohol consumption may alter the breakdown of those medications and may contribute to harmful interactions between alcohol and those medications.

![The Extracellular Matrix](image-url)

**Figure 5.1: The Extracellular Matrix**

The primary component of the MEOS is the molecule cytochrome P450, which exists in several variants. The variant most important for alcohol metabolism is cytochrome P450 2E1 (CYP2E1). Studies using liver biopsies from people who recently had been drinking alcohol found that the levels of CYP2E1 were four times higher in these subjects than in control subjects who had not been drinking alcohol. In contrast, the levels of ADH in the liver did not change following alcohol consumption. Enhanced CYP2E1 activity in response to chronic alcohol consumption (or other factors) probably contributes to the development of alcoholic liver disease. Alcoholics commonly suffer from a type of liver disease called steatohepatitis, which is an inflammation of the liver with concurrent fat accumulation in the liver. Steatohepatitis also is frequently found in people with diabetes and excessive or morbid obesity, even if they are not alcoholics. Studies have found that, in addition to breaking down alcohol, CYP2E1 also mediates condition called oxidative stress, which can cause liver cell damage. These ROS effects are exacerbated if the body's normal defenses systems against this damage - antioxidants,
such as glutathione (GSH) and vitamin E (α-tocopherol) - also are impaired. Alcohol and its metabolism have been shown to reduce the levels of both GSH and vitamin E. For example, the breakdown product of alcohol, acetaldehyde, lowers GSH levels in the liver. Furthermore, patients with cirrhosis have reduced amounts of vitamin E in the liver. Thus, alcohol metabolism through the MEOS can lead to liver damage both by generating harmful substances (the ROS) and by reducing the levels of protective substances (GSH).

2.7.5. Liver Function Test

Liver function tests (LFTs or LFss), which include liver enzymes, are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (eg. albumin); some with cellular integrity (eg. transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease (Nyblom et. al. 2004) distinguish among different types of liver disorders (Nyblom et. al. 2006) gauge the extent of known liver damage, and follow the response to treatment.

**Alanine transaminase (ALT)** Alanine transaminase (ALT), also called Serum Glutamic Pyruvate Transaminase (SGPT) or Alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. ALT rises dramatically in acute liver damage, such as viral hepatitis or paracetamol (acetaminophen) overdose. Elevations are often measured in multiples of the upper limit of normal (ULN). Reference range (Normal Values) 9 to 60 IU/L

**Aspartate transaminase (AST)** Aspartate transaminase (AST) also called Serum Glutamic Oxaloacetic Transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal
muscle and is therefore not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. Elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker. Reference range (Normal Values) 10 to 40 IU/L

**Alkaline phosphatase (ALP)** Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodelled) and elderly patients with Paget's disease. Reference range (Normal Values) 30 to 120 IU/L

**Total bilirubin (TBIL)** Bilirubin is a breakdown product of heme (a part of haemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. It does this by the following mechanism: bilirubin is taken up into hepatocytes, conjugated (modified to make it water-soluble), and secreted into the bile, which is excreted into the intestine. Increased total bilirubin causes jaundice, and can signal a number of problems:

1. **Prehepatic:** Increased bilirubin production. This can be due to a number of causes, including hemolytic anemias and internal hemorrhage.

2. **Hepatic:** Problems with the liver, which are reflected as deficiencies in bilirubin metabolism (e.g. reduced hepatocyte uptake, impaired conjugation of bilirubin, and reduced hepatocyte secretion of bilirubin). Some examples would be cirrhosis and viral hepatitis.

3. **Posthepatic:** Obstruction of the bile ducts, reflected as deficiencies in bilirubin excretion (Obstruction can be located either within the liver or in the bile duct). Reference range (Normal Values) 0.1–1.2 mg/dL.

**Direct bilirubin** The diagnosis is narrowed down further by looking at the levels of direct bilirubin. If direct (i.e. conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin, and the location of the problem is upstream of bilirubin excretion. Hemolysis, viral hepatitis, or cirrhosis can be suspected. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile
duct obstruction by gallstones or cancer should be suspected. Reference range (Normal Values) 0–0.3 mg/dL

2.8. KIDNEY

2.8.1. Anatomy

The kidneys are dark-red, bean-shaped organs. One side of the kidney bulges outward (convex) and the other side is indented (concave). There is a cavity attached to the indented side of the kidney, called the Renal Pelvis... which extends into the ureter. Each kidney is enclosed in a transparent membrane called the renal capsule... which helps to protect them against infections and trauma. The kidney is divided into two main areas... a light outer area called the renal cortex, and a darker inner area called the renal medulla. Within the medulla there are 8 or more cone-shaped sections known as renal pyramids. The areas between the pyramids are called renal columns.

![Figure 6.1: Anatomy of the kidney](image-url)
2.8.2. Biochemical Function

a) The kidneys regulate the amount and structure of fluids and electrolytes in your body. They help clear waste from cells and help in the contribution of nutrients that help form cells as well as provide stable conditions for the cells to function. They help normalize the acid-base equilibrium of the cell and also produce hormones.

b) Hydrogen ion concentration or acid-base equilibrium is vital to accurate metabolic reactions. If accurate levels are interrupted by alcohol, disturbances such as low levels of phosphates can cause hyperventilation and low acidity.

c) Within 20 minutes, alcohol can have an adverse reaction to urine flow. It can increase urine flow, causing a change in the concentration of electrolytes in the blood, in particular antidiuretic hormone which encourages the kidneys to conserve fluids. This in turn discourages water being taken back in by the body and thus increases electrolyte levels in the blood.

d) Sodium, phosphorus, magnesium and potassium are all electrolytes that are affected in a negative manner from the consumption of alcohol. All cells, mainly neurons in your brain, are dependent on levels of these electrolytes being stable. When the electrolyte levels are disrupted, your brain has difficulty regulating bodily processes and modifications in behavior occur.

e) High blood pressure can cause chronic kidney disease. Drinking alcohol may raise blood pressure to an unhealthy level. Alcohol also contains a lot of calories that can lead to weight gain -- a contributing factor to high blood pressure. Alcohol can also alter the effect of some blood pressure medications.

f) People with diabetes are at risk for chronic kidney disease. Consuming alcohol can alter the liver's ability to produce glucose. Once alcohol has entered the liver, it will not produce glucose again until all of the alcohol is cleared from the body. This can cause low glucose levels in diabetics. Alcohol also contains a lot of calories; for diabetics trying to control glucose levels with diet and exercise, this could be an issue.

2.8.3. Effect of Alcohol on Kidney

Alcohol destroys this delicate balance of the ions and water in the body by altering the filtering ability of the kidneys. Although the exact mechanisms for how alcohol
changes the kidney’s ability to function are not clearly known, the changes in ionic concentrations have been studied in humans and in animals for many years. Kidney complications are even greater if a person has also been diagnosed with liver damage due to alcohol consumption.

Urination can be induced 20 minutes after a person consumes alcohol possibly due to alcohol affecting the availability ADH. When alcohol inhibits ADH, the nephrons in the kidneys become less permeable to water, and more water travels through the ureters to the bladder. More water leaving the body affects all the concentrations of the ions in the body, negatively affecting many metabolic processes. The kind of alcohol consumed can either increase or decrease the concentrations of certain ions in the blood stream. Beer is low in dissolved nutrients. When a person drinks beer, large amounts of water enter the body; that lowers the concentration of metabolic nutrients and because of the effect of ADH impairment, an equal amount of water does not leave the body in the urine. Fluid overload in the blood stream decreases the body’s ionic concentrations and can be very dangerous, especially for advanced alcoholics who also have liver disease. The following table gives an overview of the effects of decreased ionic concentrations:

*The Effects of Decreased Ionic Concentrations*

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Phosphate</th>
<th>Magnesium</th>
</tr>
</thead>
</table>
| Effects | -Impaired mental activity.  
- Seizure in extreme cases. | - Increased thirst.  
- Hormonal imbalance promoting fluid intake. | - Decreased blood acidity resulting in a breakdown of glucose and increased metabolic activity.  
- Resulting low blood sugar. | - Possible enzyme impairment. |

When a person drinks hard alcohol (such as whiskey or vodka) the ionic concentrations can increase in the blood stream. The suppression of ADH causes more liquid to leave the body as water, and ionic concentrations left in the blood can rapidly increase as more ions (mostly sodium) are ingested with the alcohol. Alcohol can also impact the muscle cells of the body, causing them to release ions (i.e. phosphate). The effects of more ions in the blood stream impacts the water held in the cells of the body through osmosis.
Osmosis pulls the water that resides in the cells into the bloodstream to counteract the ionic imbalance. This drying effect can negatively impact the normal function of cells and organs.

**The Effects of Increased Ionic Concentrations**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Phosphate</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects</td>
<td>- Osmotic flow of water out of body cells to areas of high sodium.</td>
<td>- Osmotic flow of water out of body cells to areas of high potassium.</td>
<td>- Creates a buffer imbalance in the blood. - Increase in blood pH.</td>
<td>- Possible enzyme impairment.</td>
</tr>
</tbody>
</table>

One alcohol drink can affect the normal function of a person’s kidneys. While only severe alcoholics suffer from some of the complications, keep in mind that these ionic imbalances occur each time a person drinks.

Prolonged heavy drinking can cause kidney failure. The primary functions of kidneys are to regulate the composition and volume of the fluids and electrolytes circulating through the body. The kidneys regulate water, acid/base balance, certain hormones and minerals (calcium, potassium, sodium, etc.) in the body. Alcohol can influence or compromise the balancing functions of the kidneys, and thus can cause severe consequences on kidney function and thus the body.

After alcohol is ingested, it reaches the stomach where about 20% of the alcohol absorbs into the bloodstream, through small blood vessels. The remaining 80% of the alcohol continues to the small intestine and is absorbed there into the bloodstream.

The alcohol flows through the blood stream and is metabolized by the liver, where the alcohol is broken down by enzymes. The liver can, on average, metabolize about one standard drink (i.e. one 12 ounce bottle of beer, one 4 ounce glass of wine or 1.5 ounces of 40% alcohol) in one hour. Alcohol consumed in addition to these amounts can generally not be processed by the liver. When this happens, your blood becomes saturated and the additional alcohol makes its way to your body tissues and blood stream, until your liver can process the excess alcohol. When consumed in large amounts over a prolonged period of time, alcohol can harm virtually every part of your body. Many of the effects are reversible if alcohol consumption is subsequently controlled - other effects are permanent.
2.8.4. Mode of Mechanism of Alcohol Toxicity

A cell’s function depends not only on receiving a continuous supply of nutrients and eliminating metabolic waste products but also on the existence of stable physical and chemical conditions in the extracellular fluid bathing it. Among the most important substances contributing to these conditions are water, sodium, potassium, calcium, and phosphate. Loss or retention of any one of these substances can influence the body's handling of the others. In addition, hydrogen ion concentration (i.e., acid-base balance) influences cell structure and permeability as well as the rate of metabolic reactions. The amounts of these substances must be held within very narrow limits, regardless of the large variations possible in their intake or loss. The kidneys are the organs primarily responsible for regulating the amounts and concentrations of these substances in the extracellular fluid. In addition to their role in regulating the body’s fluid composition, the kidneys produce hormones that influence a host of physiological processes, including blood pressure regulation, red blood cell production, and calcium metabolism. Besides producing hormones, the kidneys respond to the actions of regulatory hormones produced in the brain, the parathyroid glands in the neck, and the adrenal glands located atop the kidneys. Because of the kidneys’ important and varied role in the body, impairment of their function can result in a range of disorders, from mild variations in fluid balance to acute kidney failure and death. Alcohol, one of the numerous factors that can compromise kidney function, can interfere with kidney function directly, through acute or chronic consumption, or indirectly, as a consequence of liver disease.

Gross and microscopic changes  One way in which alcohol directly affects the kidneys is by altering the form and structure of this pair of organs, as demonstrated by various animal studies. For example, in an early study on dogs (Chaikoff et. al. 1948), investigators observed several striking alterations after chronic alcohol administration. The basement membrane of the glomerulus became abnormally thickened and was characterized by cell proliferation. Further changes included enlarged and altered cells in the kidney tubules. In another study, Van Thiel and colleagues (1977) compared kidney structure and function in alcohol-fed and control rats. The alcohol-fed group experienced kidney swelling and significantly reduced kidney function; in addition, under microscopic examination, the kidneys of alcohol-fed rats were found to have cells
enlarged with increased amounts of protein, fat, and water, compared with those of the control animals. Similarly, clinicians long have noted significant kidney enlargement (i.e., nephromegaly) in direct proportion to liver enlargement among chronic alcoholic patients afflicted with liver cirrhosis. Laube and colleagues (1967) suggested that both cellular enlargement and cell proliferation contribute to such nephromegaly. In alcoholic patients with cirrhosis, these investigators reported a 33-percent increase in kidney weight, whereas they observed no appreciable kidney enlargement in alcoholic patients without cirrhosis compared with control subjects (Laube et al. 1967).

**Blood-flow changes** Normally the rate of blood flow, or perfusion, (i.e., hemodynamics) through the kidneys is tightly controlled, so that plasma can be filtered and substances the body needs can be reabsorbed under optimal circumstances. Established liver disease impairs this important balancing act, however, by either greatly augmenting or reducing the rates of plasma flow and filtration through the glomerulus. Investigators have not yet fully explained the mechanisms underlying this wide range of abnormalities, though, and have devoted little attention to alcohol’s effects on kidney hemodynamics in people who do not have liver disease. The few studies focusing on alcohol’s direct effects on perfusion in human kidneys suggest that regulatory mechanisms retain control over this component of kidney function despite alcohol consumption. Even at high blood alcohol levels, only minor fluctuations were found in the rates of plasma flow and filtration through the kidneys (Rubini et al. 1955). Additional studies are needed to confirm these observations, however. Results of subsequent studies in animal models seem to vary according to the species examined, the route and dose of alcohol administration, and the length of time after administration for which the study groups were observed. For example, some studies implied that acute alcohol consumption does not significantly change kidney hemodynamics or sodium excretion in dogs, but these studies did not extend beyond 6 hours after alcohol ingestion. In contrast, earlier studies that examined dogs for a longer period reported that a single dose of 3 grams of alcohol per kilogram of body weight (g/kg) elevated plasma volume between 10 and 26 hours following alcohol ingestion (Nicholson and Taylor 1940). Another study with dogs (Beard et al. 1965) disclosed that the effects of chronic alcohol consumption endured even longer. The investigators noted increased plasma and extracellular fluid volume 1 week after chronic
alcohol ingestion, and these volume expansions persisted for the remaining 7 weeks of the study. Similar alterations have been found in body fluid volumes among chronic alcoholic patients.

**Effects on fluid and electrolyte balance** One of the main functions of the kidneys is to regulate both the volume and the composition of body fluid, including electrically charged particles (i.e., ions), such as sodium, potassium, and chloride ions (i.e., electrolytes). However, alcohol’s ability to increase urine volume (i.e., its diuretic effect) alters the body’s fluid level (i.e., hydration state) and produces disturbances in electrolyte concentrations. These effects vary depending on factors such as the amount and duration of drinking, the presence of other diseases, and the drinker’s nutritional status.

**Fluid** Alcohol can produce urine flow within 20 minutes of consumption; as a result of urinary fluid losses, the concentration of electrolytes in blood serum increases. These changes can be profound in chronic alcoholic patients, who may demonstrate clinical evidence of dehydration. As most investigators now agree, increased urine flow results from alcohol’s acute inhibition of the release of antidiuretic hormone (ADH), a hormone also known as vasopressin, which normally promotes the formation of concentrated urine by inducing the kidneys to conserve fluids. In the absence of ADH, segments of the kidney’s tubule system become impermeable to water, thus preventing it from being reabsorbed into the body. Under these conditions, the urine formed is dilute and electrolyte concentration in the blood simultaneously rises. Although increased serum electrolyte concentration normally activates secretion of ADH so that fluid balance can be restored, a rising blood alcohol level disrupts this regulatory response by suppressing ADH secretion into the blood. Interestingly, age makes a difference in how rapidly the body escapes alcohol’s ADH-suppressive effect. People older than age 50 overcome suppression of ADH more quickly than their younger counterparts do, despite reaching similar serum electrolyte concentrations after alcohol consumption. In older people, ADH levels sharply increase following alcohol intake, perhaps in part because sensitivity to increased electrolyte concentration is enhanced with age. It is not known whether chronic alcoholic patients experience a similar difference in the ADH response as they age, however.
**Sodium** The serum sodium level is determined by the balance of fluid in relation to that of sodium: Not enough fluid in the body results in a sodium concentration that is too high (i.e., hypernatremia), whereas excessive amounts of fluid produce a sodium concentration that is too low (i.e., hyponatremia). Hyponatremia does not constitute merely a biochemical abnormality but most likely has clinical consequences as well (e.g., impaired mental activity, neurological symptoms, and, in extreme instances, seizures). “Beer drinkers’ hyponatremia” is a syndrome that appears to result from an intake of excessive fluid in the form of beer. Hilden and Svendsen (1975) observed hyponatremia in five patients who drank at least 5 liters of beer per day (L/d) without any other nourishment. (For comparison, a person’s normal fluid intake averages a little more than 2 L/d.) Because beer contains few dissolved substances (i.e., solutes), such as sodium, these patients apparently lacked a sufficient quantity of solutes to stimulate the kidneys to eliminate excess fluid. Although fluid overload—not alcohol itself—is considered the major contributor to beer drinkers’ hyponatremia, alcohol does appear to directly influence the kidney’s handling of sodium and other electrolytes, potentially resulting in hypernatremia. In a study by Rubini and colleagues (1955), subjects who consistently drank about 4 ounces (oz) of 100-proof bourbon whiskey experienced decreased sodium, potassium, and chloride excretion (i.e., increased retention of solutes). Although some exceptions exist, several historical studies have reported similar modest reductions in sodium and potassium excretion following alcohol use. In general, however, neither acute nor chronic alcohol consumption directly causes significant changes in serum sodium concentrations, although impaired sodium excretion is a frequent complication of advanced liver disease.

**Potassium** Normally the kidneys are a major route of potassium ion excretion and serve as an important site of potassium regulation. Alcohol consumption historically has been found to reduce the amount of potassium excreted by the kidneys (e.g., Rubini *et al.* 1955), although the body’s hydration state may help determine whether potassium excretion will increase or decrease in response to alcohol. Levels of potassium, like those of sodium, also can affect the way the kidneys handle fluid elimination or retention. In addition, potassium depletion has been proposed to exacerbate hyponatremia through any of several mechanisms (Epstein 1992): For example, potassium losses may stimulate...
ADH activity, thereby increasing the amount of fluid reabsorbed and causing the body’s sodium concentration to decrease as a result. Alternatively, potassium losses may increase thirst, also through hormonal mechanisms, thereby promoting increased fluid intake.

**Phosphate** Low blood levels of phosphate commonly occur acutely in hospitalized alcoholic patients, appearing in more than one-half of severe alcoholism cases. Indeed, when the condition does not appear, clinicians treating alcoholic patients should suspect that another problem is masking the recognition of low phosphate levels, such as ongoing muscle dissolution, excess blood acidity (i.e., acidosis), inadequate blood volume, or kidney failure. Several mechanisms may contribute to abnormally low phosphate levels (i.e., hypophosphatemia). Simply lacking an adequate amount of phosphate in the diet is one possible reason for phosphate deficiency. For severely alcoholic patients who eat poorly, such a nutritional deficit may be an important contributor to hypophosphatemia. Another potential cause of hypophosphatemia in alcoholic patients is hyperventilation, which can occur during alcohol withdrawal. Prolonged rapid, shallow breathing results in excessive loss of carbon dioxide and decreased blood acidity (i.e., alkalosis), which in turn activates an enzyme that enhances glucose breakdown. In glucose breakdown, phosphate becomes incorporated into various metabolic compounds, ultimately lowering blood levels of phosphate. As the rate of glucose breakdown increases, profound hypophosphatemia potentially can result. Insulin administration also can lead to mild hypophosphatemia, because it decreases cellular acidity. Although insulin more likely plays a contributory, rather than principal, role in producing hypophosphatemia in alcoholic patients, there are clinical implications to consider. Both glucose and amino acids are powerful triggers for insulin release, and hospitalized alcoholic patients frequently receive intravenous fluids containing these nutrients. Physicians thus should be prepared to respond if hypophosphatemia develops. A similar concern applies to another treatment that may lead to hypophosphatemia: overfeeding patients beyond normal nutrient requirements in an attempt to replace dietary deficiencies. Alcoholic patients also may develop low blood levels of phosphate by excreting too much of this ion into their urine. Typically, chronic alcoholic patients are losing up to 1.5 g/d of phosphate through their urine when they have reached the point of being sick enough to
accept hospitalization. (For comparison, a normal healthy person excretes 0.7 to 0.8 g/d.) Over the next several days of hospitalization, these patients often excrete virtually no phosphate in their urine; simultaneously, their blood phosphate levels dip to low levels before returning to normal. The combination of low phosphate excretion and low blood levels indicates that phosphate is simply being shifted from the bloodstream into body cells, implying that kidney dysfunction is not a likely cause of phosphate wasting in this case. Alcohol can induce abnormally high phosphate levels (i.e., hyperphosphatemia) as well as abnormally low levels. In fact, hyperphosphatemia often precedes hypophosphatemia. Alcohol consumption apparently leads to excessive phosphate levels by altering muscle cell integrity and causing the muscle cells to release phosphate. This transfer of phosphate out of muscle cells and into the bloodstream results in an increased amount of phosphate passing through the kidneys’ filtering system. In response, reabsorption of phosphate diminishes and excretion in urine increases in an effort to return blood levels of this ion to normal.

**Magnesium** Chronic alcoholism is the leading cause of low blood levels of magnesium (i.e., hypomagnesemia) in the United States (Epstein 1992). Often it occurs simultaneously with phosphate deficiencies, also frequently encountered among alcoholic patients. Hypomagnesemia responds readily to magnesium supplementation treatment, however. Several alcohol-related mechanisms can result in hypomagnesemia. Studies historically have shown that alcohol consumption markedly increases magnesium excretion in the urine and may affect magnesium levels in other ways as well. For example, when rats are given alcohol, they also require significant magnesium in their diets, suggesting that alcohol disrupts absorption of this nutrient from the gut. Investigators have speculated that alcohol or an intermediate metabolite directly affects magnesium exchange in the kidney tubules (Epstein 1992).

**Calcium** Early studies showed that alcohol consumption markedly increases calcium loss in urine. In severely ill alcoholic patients, low blood levels of calcium occur about as often as low blood levels of phosphate and can cause convulsions or potentially life-threatening muscle spasms when respiratory muscles are involved. Alcoholic patients with liver disease often have abnormally low levels of a calcium-binding protein, albumin, and also may have impaired vitamin D metabolism; either of these two factors
could result in reduced blood levels of calcium (i.e., hypocalcemia). Muscle breakdown and magnesium deficiency are other potential causes of hypocalcemia in alcoholic patients. A direct effect of alcohol in reducing calcium levels is suggested by at least one experimental study: Dogs became hypocalcemic after administration of alcohol above a critical threshold amount of approximately 1 g/kg (Money et. al. 1989).

**Body fluid volume and blood pressure** chronic alcohol consumption may cause both fluid and solutes to accumulate, thereby increasing the overall volume of body fluids. In turn, such expansion of body fluid volume can contribute to high blood pressure, a condition often seen among chronic alcoholic patients. The association between increased blood pressure and alcohol consumption has been recognized at least since 1915, when Lian reported the prevalence of high blood pressure (i.e., hypertension) in relation to the drinking habits of French army officers. More recent studies have substantiated this link. For example, in the large scale Kaiser-Permanente study, in which blood pressure measurements and alcohol histories were obtained from more than 80,000 men and women, the association between blood pressure and drinking was found to be independent of age, sex, ethnicity, weight, smoking habit, and social class (Klatsky et. al. 1977). Clinical studies of hypertensive patients have demonstrated that reducing alcohol intake lowers blood pressure and resuming consumption raises it. Although the mechanisms responsible for these effects have not been established, an experimental study by Chan and Sutter (1983) offers some insight. In this study, male rats given 20-percent alcohol in their drinking water for 4 weeks experienced decreased urinary volume and sodium excretion as well as increased blood concentrations of hormones that raise blood pressure by constricting blood vessels. The results of this study suggest that alcohol’s influence on blood pressure may be attributable, at least in part, to its effects on the production of hormones that act on the kidneys to regulate fluid balance or that act on blood vessels to constrict them.

**Acid-base balance effects** Most of the metabolic reactions essential to life are highly sensitive to the acidity (i.e., hydrogen ion concentration) of the surrounding fluid. The kidneys play an important role in regulating acidity, thereby helping determine the rate at which metabolic reactions proceed. Alcohol can hamper the regulation of acidity, thus affecting the body’s metabolic balance. One example of an alcohol-related acid-base
disturbance already has been mentioned in relation to low levels of phosphate (i.e., respiratory alkalosis resulting from hyperventilation during alcohol withdrawal). Other acid-base disturbances are possible as a result of excessive alcohol consumption. These disturbances increase the kidneys’ workload in restoring acid-base balance through formation of an acidic or basic (i.e., alkaline) urine. For instance, the opposite of respiratory alkalosis can occur when a person becomes extremely intoxicated. Because alcohol is a central nervous system depressant, it may slow the rate of breathing as well as reduce the brain’s respiratory center’s sensitivity to carbon dioxide levels. As a result, excess carbon dioxide accumulates, and the body’s acid level subsequently increases. Respiratory acidosis is rare but carries an ominous prognosis when it occurs. Excess blood acidity in alcoholic patients more often results from severe elevations of a product of glucose metabolism (i.e., lactate), which can be induced by alcohol consumption as well as other factors. A potentially serious condition known as alcoholic ketoacidosis is another disorder associated with abnormally high blood acidity. Characterized by an abnormal accumulation of ketone bodies, which are substances manufactured in the liver and used as reserve fuels for muscle and brain tissue, ketoacidosis also is a complication of uncontrolled diabetes and starvation. Typically, alcoholic ketoacidosis occurs in chronic alcohol abusers following a severe binge in which they consume alcoholic beverages and nothing else over several days. Certain people appear to be particularly prone to alcoholic ketoacidosis and may develop the condition repeatedly, but the reason for their susceptibility remains unknown (Epstein 1992). Additional causes of nonrespiratory acidosis include drinking nonbeverage alcohol (e.g., antifreeze or wood alcohol), which alcoholics sometimes resort to consuming when beverage alcohol is unavailable; aspirin overdose; and administration of paraldehyde, a sedative used for alcohol withdrawal. Despite the multiple possible causes of acidosis, disturbances in acid-base balance are more frequently manifested as low acidity (i.e., alkalosis). Alkalosis was present in 71 percent of patients with established liver disease in 11 studies, and respiratory alkalosis was the most common disturbance in 7 of the studies (Oster and Perez 1996). If an acute alcoholic binge induces extensive vomiting, potentially severe alkalosis may result from losses of fluid, salt, and stomach acid. Like the kidneys, the liver plays an important role in maintaining acid-base balance. Liver
diseases—including alcohol-induced liver problems— disrupt this function and can contribute directly or indirectly to a wide range of acid-base disturbances.

**Regulatory effects** To keep the kidneys functioning optimally and to maintain functional stability (i.e., homeostasis) in the body, a variety of regulatory mechanisms exert their influence. Alcohol can perturb these controls, however, to a degree that varies with the amount of alcohol consumed and the particular mechanism’s sensitivity. As an example, Puddey and colleagues (1985) evaluated the effects of hormones that regulate kidney function. Their results show not only how alcohol disruptions homeostasis but also how the body reacts to restore it. Following moderate alcohol consumption— about 24 oz—of nonalcoholic beer with 1 milliliter of alcohol per kilogram of body weight added, the investigators noted several effects. Alcohol-induced urination reduced the subjects’ plasma volume, resulting in an increased concentration of plasma sodium. In addition, the subjects’ blood pressure and plasma potassium concentration decreased. These changes in fluid volume, electrolyte balance, and blood pressure may have stimulated the activity of hormones to return body fluid volume and composition back to normal, which occurred soon after consumption. Alcohol consumption also is known to induce a state of low blood sugar (i.e., hypoglycemia) and activate the portion of the nervous system that coordinates the body’s response to stress (i.e., the sympathetic nervous system). Both of these factors affect hormones that regulate kidney function, just as changes in fluid volume and electrolyte balance do.

**Indirect effects** Physicians have recognized an interrelationship between kidney and liver disorders at least since the time of Hippocrates. Although a disorder in one organ can complicate a primary problem in the other (or a pathological process may involve both organs directly), kidney dysfunction complicating a primary disorder of the liver (e.g., cirrhosis) is the most clinically significant scenario. Frequently, such kidney dysfunction results from liver problems related to alcohol. In fact, most patients in the United States diagnosed with both liver disease and associated kidney dysfunction are alcohol dependent (Epstein 1992). Three of the most prominent kidney function disturbances that arise in the presence of established liver disease are impaired sodium handling, impaired fluid handling, and acute kidney failure unexplained by other causes (i.e., hepatorenal syndrome).
Impaired Sodium Handling Patients with alcohol-induced liver cirrhosis show a great tendency to retain salt (i.e., sodium chloride), and their urine frequently is virtually free of sodium. A progressive accumulation of extracellular fluid results, and this excess fluid is sequestered primarily in the abdominal region, where it manifests as marked swelling (i.e., ascites). In addition, excess fluid accumulates in spaces between cells, clinically manifested as swelling (i.e., edema) of the lower back and legs. As long as cirrhotic patients remain unable to excrete sodium, they will continue to retain the sodium they consume in their diet. Consequently, they will develop increasing ascites and edema and experience weight gain. In some cases, vast amounts of abdominal fluid may collect, occasionally more than 7 gallons (Epstein 1996). Rigorously limiting sodium intake, which is the first step in treating ascites, will halt fluid retention. Such sodium restriction alone may bring about a spontaneous increase in urine flow and relieve ascites, but how often this response occurs and in which patients is unknown and unpredictable. Other treatment options include various diuretic agents and, when ascites stubbornly persists, aspiration of the excess abdominal fluid. Many sodium retaining patients who receive large quantities of sodium-free water can excrete copious amounts of dilute urine, thus demonstrating that the main kidney dysfunction associated with their ascites is an impaired ability to excrete sodium, not water. The events leading to abnormal sodium handling in patients with cirrhosis are complex and controversial, however. Investigators have advanced several theories suggesting the involvement of a constellation of hormonal, neural, and hemodynamic mechanisms (Epstein 1996; Laffi et. al. 1996).

Impaired Fluid Handling In many patients with liver cirrhosis, the kidneys’ ability to create dilute urine is compromised, leading to a state of abnormally low sodium concentration (i.e., hyponatremia). In hyponatremic patients, the amount of fluid retained by the kidneys is disproportionately greater than the amount of sodium retained. In other words, the kidneys’ ability to excrete excess fluid by way of dilute urine is impaired, and too much fluid is reabsorbed. Hyponatremia probably is the single most common electrolyte disturbance encountered in the management of patients with cirrhosis of the liver (Vaamonde 1996). This abnormality may reflect the severity of liver disease, but the available data do not allow correlation of kidney impairment with the degree of clinical signs of liver disease, such as ascites or jaundice. A compromised diluting ability has
important implications for the management of patients with advanced liver disease. Restricting the fluid intake of hyponatremic patients eventually should restore a normal fluid balance; unfortunately, this restriction may be difficult to implement. Patients frequently fail to comply with their physician’s orders to limit their fluid intake. Furthermore, clinicians sometimes overlook the fact that fluids taken with medications also must be restricted for these patients and mistakenly bring pitchers of juice or water to their bedsides. The difficulties in successfully managing dilutional hyponatremia have resulted in the recent emergence of a promising class of new drugs to treat this abnormality. Specifically, drugs known as arginine vasopressin antagonists are being developed to inhibit ADH at the cell receptor level. These new drugs should dramatically facilitate treatment of cirrhotic patients with impaired fluid handling.

**Hepatorenal Syndrome** Hepatorenal syndrome may appear in patients afflicted with any severe liver disease, but in the United States, studies most often have identified alcoholic cirrhosis as the underlying disorder. Major clinical features of hepatorenal syndrome include a marked decrease in urine flow, almost no sodium excretion and, usually, hyponatremia and ascites. Blood urea nitrogen (BUN) levels and serum concentrations of the waste product creatinine are somewhat elevated, but rarely to the degree seen in patients with end-stage kidney failure when kidney disease is the primary disorder. Judgments based on such relatively modest BUN and serum creatinine increases often underestimate kidney dysfunction in patients with hepatorenal syndrome, however, because malnourished cirrhotic patients tend to have low levels of urea and creatinine. Although hepatorenal syndrome often ensues after an event that reduces blood volume (e.g., gastrointestinal bleeding), it also can occur without any apparent precipitating factor. Some observers have noted that patients with cirrhosis frequently develop hepatorenal syndrome following hospital admission, possibly indicating that a hospital-related event can trigger the syndrome. Regardless of the precipitating factor, patients who develop kidney failure in the course of alcoholic cirrhosis have a grave prognosis. Substantial evidence exists to support the concept that kidney failure in hepatorenal syndrome is not related to structural damage and is instead functional in nature. For example, almost 30 years ago, Koppel and colleagues (1969) demonstrated that kidneys transplanted from patients with hepatorenal syndrome are capable of resuming normal
function in recipients without liver disease. In addition, Iwatsuki and colleagues (1973) and Gonwa and Wilkinson (1996) documented the return of normal kidney function in hepatorenal syndrome patients who receive liver transplants. Indeed, liver transplantation is one of two options available today for treating hepatorenal syndrome. Gonwa and Wilkinson (1996) reported a 4-year survival rate of 60 percent in hepatorenal syndrome patients who received a liver transplant, which constitutes a major step forward, considering the previous uniformly fatal course of the disease. Another current treatment option is known as transjugular intrahepatic portosystemic shunt (TIPS), in which a bypass (i.e., shunt) between two veins inside the liver (i.e., the hepatic vein and the portal vein) is created by way of the jugular vein. Obstructions in the liver of cirrhotic patients increase pressure in the portal vein, and this effect is thought to contribute to many kidney complications. The TIPS technique was developed as a means to reduce pressure in the portal circuit and offers several advantages: Because the shunt can be inserted under local anesthesia, TIPS avoids postoperative complications associated with surgery. In addition, TIPS does not alter the anatomy of the blood vessels outside the liver, an important consideration for potential liver-transplant candidates. The less invasive nature of TIPS makes it an attractive option for treating hepatorenal syndrome, and preliminary results show that the procedure is effective (Somberg 1996). Currently, a clinical trial sponsored by the National Institutes of Health is investigating the effects of TIPS on the treatment of ascites and improvement of kidney function.

**2.8.5. Kidney Function Test**

The kidneys are the filtering devices of blood. The kidneys remove waste products from metabolism such as urea, uric acid, and creatinine by producing and secreting urine. Urine may also contain sulfate and phenol waste and excess sodium, potassium, and chloride ions. The kidneys help maintain homeostasis by regulating the concentration and volume of body fluids. For example, the amount of $H^+$ and $HCO_3^-$ secreted by the kidneys controls the body's pH.

**2.9. DRUG METABOLISM**

Mostly drugs are metabolized by the liver. Hepatic metabolism usually increases the hydrophilicity of drugs and therefore their ability to be excreted. There is some inactive part of drug which has to be converted to their active forms as a result of processing in
the liver. The drug metabolism proceeds in two phases. Phase I – polarity of the drug is increased by oxidation or hydroxylation catalyzed by a family of microsomal cytochrome P450 oxidases. Phase II- cytoplasmic enzyme conjugates the functional groups introduced in the 1st Phase reactions most of them by glucronidation or sulfation (cytochrome P450 enzymes are heme containing proteins).

2.10. ANCIENT PLANTS USED AS MEDICINE

Few herbal remedies have conclusively demonstrated any positive effect on humans, possibly due to inadequate testing (Ernst 2007). Many of the studies cited refer to animal model investigations or in-vitro assays and therefore cannot provide more than weak supportive evidence.

Aloe Vera has traditionally been used for the healing of burns and wounds (Maenthaisong et. al. 2007). A systematic review (from 1999) states that the efficacy of aloe vera in promoting wound healing is unclear, while a later review (from 2007) concludes that the cumulative evidence supports the use of aloe Vera for the healing of first to second degree burns (Maenthaisong et. al. 2007; Vogler and Ernst 1999). Agaricus blazei mushrooms may prevent some types of cancer (Kimura et. al. 2004).

Artichoke (Cynara cardunculus) may reduce production cholesterol levels according to in vitro studies (Gebhardt 1998) and a small clinical study. Boophone (Boophone disticha) this highly toxic plant has been used in South African traditional medicine for treatment of mental illness (Stafford et. al. 2008). Research demonstrates in vitro and in vivo effect against depression (Pedersen et. al. 2008 and Neergaard et. al. 2009).

Calendula (Calendula officinalis) has been used traditionally for abdominal cramps and constipation (Bashir et. al. 2006). In animal research an aqueous-ethanol extract of Calendula officinalis flowers was shown to have both spasmolytic and spasmogenic effects, thus providing a scientific rationale for this traditional use (Bashir et. al. 2006). There is "limited evidence" that calendula cream or ointment is effective in treating radiation dermatitis. Cranberry (Vaccinium oxycoccos) may be effective in treating urinary tract infections in women with recurrent symptoms (Jepson and Craig 2008). Echinacea (Echinacea angustifolia, Echinacea pallida, Echinacea purpurea) extracts may limit the length and severity of rhinovirus colds; however, the appropriate
dosage levels, which might be higher than is available over-the-counter, require further research (Shah et. al. 2007). Feverfew (Chrysanthemum parthenium) is sometimes used to treat migraine headaches. Although many reviews of Feverfew studies show no or unclear efficacy, a more recent RTC showed favorable results (Silberstein 2005). Feverfew is not recommended for pregnant women as it may be dangerous to the fetus (Yao et. al. 2006). Gawo (Faidherbia albida), a traditional herbal medicine in West Africa, has shown promise in animal tests (Tijani 2008). Garlic (Allium sativum) may lower total cholesterol levels (Ackerman 2001). German Chamomile (Matricaria chamomilla) has demonstrated antispasmodic, anxiolytic, antiinflammatory and some antimutagenic and cholesterol-lowering effects in animal research. In vitro chamomile has demonstrated moderate antimicrobial and antioxidant properties and significant antiplatelet activity, as well as preliminary results against cancer. Essential oil of chamomile was shown to be a promising antiviral agent against herpes simplex virus type 2 (HSV-2) in vitro (Koch et. al. 2008). Ginger (Zingiber officinale), administered in 250 mg capsules for four days, and effectively decreased nausea and vomiting of pregnancy in a human clinical trial (Ozgoli et. al. 2009). Grapefruit (Naringenin) components may prevent obesity. Green tea (Camelia sinensis) components may inhibit growth of breast cancer cells and may heal scars faster (Zhang et. al. 2006). Purified extracts of the seeds of Hibiscus sabdariffa may have some antihypertensive, antifungal and antibacterial effect. Toxicity tested low except for an isolated case of damage to the testes of a rat after prolonged and excessive consumption (Ali et. al. 2005).

Lemon grass (Cymbopogon citratus), administered daily as an aqueous extract of the fresh leaf, has lowered total cholesterol and fasting plasma glucose levels in rats, as well as increasing HDL cholesterol levels. Lemon grass administration had no effect on triglyceride levels (Mahdi et. al. 2006).

Meadowsweet (Filipendula ulmaria, Spiraea ulmaria) can be used for a variety of anti-inflammatory and antimicrobial purposes due to presence of salicylic acid. It is effective for fevers, inflammations, pain relief, ulcers and bacteriostatic listed as therapeutical in 1652 by Nicholas Culpeper. In 1838, salicylic acid was isolated from the plant. The word Aspirin is derived from spirin based on Meadowsweet's synonym name Spiraea ulmaria (Mahdi et. al. 2006). Milk thistle (Silybum marianum) extracts have
been recognized for many centuries as "liver tonics." Research suggests that milk thistle extracts both prevent and repair damage to the liver from toxic chemicals and medications (Szilard et. al. 1988).

*Morinda citrifolia* (noni) is used in the Pacific and Caribbean islands for the treatment of inflammation and pain. Human studies indicate potential cancer preventive effects (Wang et. al. 2009). *Nigella sativa* (Black cumin) has demonstrated analgesic properties in mice. The mechanism for this effect, however, is unclear. *In vitro* studies support antibacterial, antifungal, anticancer, anti-inflammatory and immune modulating effects (Hajhashemi et. al. 2004). However few randomized double blind studies have been published. *Ocimum gratissimum* (Martin and Ernst 2003) and tea tree oil can be used to treat acne. Pawpaw can be used as insecticide (killing lice, worms) (Cappello 2007). Peppermint oil may have benefits for individuals with irritable bowel syndrome (Liu et. al. 1997). Phytolacca or Pokeweed can be applied topically or taken internally. Topical treatments have been used for acne and other ailments. It is used as a treatment for tonsillitis, swollen glands and weight loss. Pomegranate contains the highest percentage of ellagitannins of any commonly consumed juice. Punicalagin, an ellagitannin unique to pomegranate, is the highest molecular weight polyphenol known (Heber 2008).

There are numerous plants and traditional formulations available for the treatment of liver diseases (Rai 1994 and Schuppan et. al. 1999). About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations (Sharma et. al. 1991). However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficiency (Subramonium et. al. 1999). Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated (Schuppan et. al. 1999).

The medicinal plants contain several phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins,
enzymes, minerals, etc. These phytochemicals possess antioxidant activities, which prevent or can be used in the treatment of many diseases. Herbal drugs are also known to have good immunomodulatory properties. These act by stimulating both non-specific and specific immunity.

Hepatoprotective activity of the alcoholic extract (95%) of the dried leaves of *Cassia alata* (ECA) was studied against Paracetamol induced hepatic injury in albino rats. Pretreatment of the ECA reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). Histopathological observations also revealed that pretreatment with ECA protected the animals from paracetamol induced liver damage. The results indicate that the leaves of *C.alata* possess the hepatoprotective activity. This property may be attributed to the flavonoids present in the leaves of *C.alata* (Ernst 2007).

### 2.11. PLANTS METABOLITES AGAINST HEPATOTOXICITY

The medicinal plants contain several phytochemicals such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals, etc. These phytochemicals possess antioxidant activities, which prevent or can be used in the treatment of many diseases. Herbal drugs are also known to have good immunomodulatory properties. These act by stimulating both non-specific and specific immunity. *Cassia alata* Linn. (*Leguminoseae*) is a shrub found throughout India, which is traditionally used by the tribes and native medicinal practitioners for the treatment of various ailments including asthma, ringworm, skin diseases, liver disorders and rheumatism. Literature review reveals that the plant possesses antiplasmodial, antimicrobial, anti-inflammatory, larvicidal, antimitogenic, antifungal, analgesic and hypoglycemic activities (Moriyama *et. al.* 2003; Somchit *et. al.* 2003 and Villasenor 2002). The plant contains flavanoids, glycosides, tannins, phenolic compounds, sterols and terpenoids (Moriyama *et. al.* 2003 and Yadav and Kalidhar 1994). The flower heads of *Sphaeranthus indicus* Linn (*Asteraceae*) a traditional Indian medicinal plant is commonly used to nourish and improve the liver conditions. *Sphaeranthus indicus* Linn. (*Asteraceae*) commonly in Hindi known, as Gorakhmundi is an annual spreading herb,
distributed throughout the plains and wetlands of India, Sri Lanka and Australia (Kirtika and Basu 1975 and Warrier et. al. 1996)

The entire plant is reportedly used in the ayurvedic system of medicines in the treatment of epilepsy and mental disorders (Pande and Chunekar 1999). It reportedly used to cure piles, hepatitis (Paranjape 2001) and have protection against immunosuppression (Bafna and Mishra 2006). Literature reports on the ariel parts of this plant revealed the presence of an essential oil glucosides, and eudesmanoids (Balsas 1959) an alkaloid sphaeranthine and an isoflavone 5,4'4'-dimethoxy-3'-prenylbiochanin 7-o-β-galctoside with some interesting sesquiterpene (Gupta et. al. 1967) and a new flavones glycoside from the stem have been isolated from this herb.

2.12. COMMON WILD EDIBLE PLANTS OF UTTARAKHAND HIMALAYA

*Paspalum scrobiculatum*

It belongs to family *Poaceae*. It is commonly known as Koda. It is distributed throughout India. It is an annual grass, culms upto 90 cm in height, tufted on a very short rhizome, leafy from the base upwards; leaves 2- ranked, 15- 45 cm long, glabrous or sometimes softly hairy, ligule short, membranous, spikelets. Suborbicular, normally 2-ranked, glumes glabrous, upper lemma and palea crustaceous, palea with wide membraneous auricles at the base; grain biconvex, shinning, deep purple with a conspicuous ridge along the margin, tightly enclosed in the hardened lemma and palea. The parts which are used as an edible are grains.

The grains are sweet, bitter, astringent, cooling, constipating, diuretic, sedative, alexeteric, styptic and tonic. They are useful in ulcers, flatulence, strangury, diarrhea, hallucination, inflammation, hepatopathy, haemorrhages, vitiated conditions of pitta, burning sensation and general debility. The root of this plant is used in painfull urination and in eye diseases (Nazir et. al. 2010). The stem is useful for corneal opacity (Warrier et. al. 1996).
Plate 1.1 *PASPALUM SCROBICULATUM*
**Rumex nepalensis Spreng.**

Polygonaceae is a cosmopolitan family containing approximately 1,200 species in 48 genera (Freeman and Reveal 2005). They are group of morphologically different herbs, shrubs, small trees or climbers characterized by simple leaves with ochrear stipules, unilocular ovary and endospermic seeds (Hutchinson and Dadzie 1954; Brummitt 1992). The *Rumex* (polygonaceae) genus comprises several species of which leaves and roots have been used in traditional medicine for inflammation, blood purification and constipation (Newall *et al.* 1996). Because of their high oxalic acid content, they have been implicated in oxalic intoxication, mainly in children (Newall *et al.* 1996).

The growing interest in many *Rumex* species has led to the study of their biological activities, namely, the effect of *Rumex acetosa* in body weight and serum levels of amino acids and minerals (Ladeji *et al.* 1995), the psychopharmacological and purgative (Ghosh *et al.* 2003) effects of *Rumex nepalensis*, the antioxidant and cytotoxic agents from *Rumex patientia* (Demirezer *et al.* 2001), the antifertility action of *Rumex steudelii* (Gebrie *et al.* 2004), the antimicrobial and anti-inflammatory activities of *Rumex nervosus* and *Rumex abyssinicus* (Getic *et al.* 2002), the antidiarrheal effect of *Rumex maritimus* (Rouf *et al.* 2002), and the antiviral activity of *Rumex bequaertii* (Cos *et al.* 2002).

Phytochemicals, such as phenolic compounds and organic acids, are known to influence the organoleptic properties of plant foods, namely, fruits and vegetables (Vaughan and Geissler 1997), and have been successfully used in their quality control (Sousa *et al.* 2005). It has been well-recognized that significant health risks and benefits are associated with dietary food choices. This association is often attributed to the antioxidants present in fruits and vegetables, such as phenolic compounds and organic acids, which prevent oxidative damage (Silva *et al.* 2004; Pulido *et al.* 2000). Plant species are good sources of these antioxidants and may have relevance in health preservation, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Pulido *et al.* 2000; Tseng *et al.* 1997).

Several phenolic compounds, namely, phenolic acids and flavonoids (Hasan *et al.* 1994; Trichopoulou *et al.* 2000; Saleh *et al.* 1993), and organic acids (Newall *et al.* 1996) have been described in several *Rumex* species. In addition, *Rumex* species are known for
the presence of anthraquinones, which may also act as antioxidants (Hasan et. al. 1994; Saleh et. al. 1993). The root of *Rumex nepalensis* is purgative (Chopra et. al. 1986; Manandhar 2002). It is used as a substitute of Rheum spp (Chopra et. al. 1986). A strong decoction is applied to dislocated bones. Root paste is applied externally to relieve headaches. Plant decoction is used to alleviate body pain (Manandhar 2002).

*Rumex nepalensis* D.Don./ Khatura, jungali palak/Herb/Leaves- leaf extract is antiseptic and used to stop bleeding. It is also used against allergy caused by leaves of *Acasia nilotica* (Ahmed et. al. 2004).

*Rumex nepalensis*, Hook (Chenopodiaceae)/Vernacular name- Payoom- This green spinach is somewhat different in both its external appearance (morphology) and taste from the locally cultivated. It is a tall, erect, robust, annual/perennial herb, up to 1 meter in height. Stems are glabrous; leaves are ovate/elliptic-ovate, entire with cordate base. Flowers are bisexual, arranged in whorls in long terminal racemes, pink coloured. Habitat is particularly around moist places near to water sources. Fresh soft leaves of *Rumex* are collected before flowering and fruiting on the plant from June-Oct. Seeds are collected at the end of the life cycle of the plant. This green leafy vegetable *Rumex* is cooked fresh and not dried for its preservation for the months when there is shortage of green vegetables.

Cure- Anaemia. It has high iron content same as the local spinach of plains. This vegetable is provided to anemic, weak and pregnant women, as they need more iron content in their diet.
Plate 1.2 RUMEX NEPALENSIS
Diaplazium esculentum Retz.

It belongs to family Dryopteridaceae. It is commonly known as Lingura. It is distributed throughout Uttarakhand Himalayan. It is a local variety of fern of about 1m height, with bipinnate fronds, sori are present in two oblique rows in the segments, infusion of the frond is linear. Commonly grows in moist and shady places. Young fronds are used as green vegetables and also used as salad or cooked as vegetables (Upreti et al. 2009) while; mature fronds are used as fodder. Juvenile fronds of the plant are plucked by the women folk of the area. As they are experienced in separation and collection of the fronds as the edible fern species grows all along with poisonous ferns locally known as “Jakh Lingra”. The fronds are boiled and fried in oil with jakhiya (Cleome viscosa) after removing the red petiolar hairs. The matured fern leaf termed as – “Una” grass is used as cattle bedding from the month of June to October/November. During these six months this fern along with few other ferns is the major cattle bedding for the livestock of the area.

The rhizomes are kept in the granaries to check them from insect and pests. It is also used as a perfect medicine against constipation.
Plate 1.3 DIAPLAZIUM ESCULENTUM
Dioscorea bellophylla Voigh.

This plant is commonly called as “tarur” belongs to Dioscoreaceae family. It is common throughout India ascending upto 1828 m in the Himalayas. It is a twining herb with glabrous stem. The leaves are alternate, ovate- triangular to suborbicular with a deeply cordate base, basal lobes rounded, simple, glabrous and 10-20 cm* 8-15 cm. Male flowers are in raceme- like, pendent, axillary, solitary of fascicled, simple or paniculate spikes of 5.0-8.0 cm length. Female flowers are in pendulous fascicled spikes of 10-20 cm length. The capsules are oblong, winged and 2.0- 2.5 cm* 1.0-1.5 cm. The seeds are winged at the base.

Flowering is during July-Sep. and fruiting in Dec- March.

The important ethnovetenary use is to cure dermatitis (Pande et. al. 2007). Also it is used in washing purpose. Tubers are used as detergents by the local communities (Mehta and Bhatt 2007). Tubers are crushed with the help of a stone and rubbed over the wet clothes; its lather removes the dirt of the clothes. Raw tubers are consumed as a food (Abdul et. al. 2008)
Plate 1.4 *Dioscorea bellophylla*
2.13. STUDIED PLANTS

*Girardinia heterophylla/diversifolia Fries.*

This plant is commonly called as “bicchhu ghas” belongs to Urticaceae family. This is a much despised plant in the hills of north India due to be very virulent stinging hairs. The plant grows to heights of 3 or 4 feet and is often used as fencing to keep out cattle. The popular hindi name Bichchhoo means scorpion. Indeed the itch produced by the plant, which in milder doses is that of many red ants, and with extensive contact can be like that of bees or scorpion stings, and may need anti-allergic medication. The itch is produced from the formic acid contained in the oil glands under the stinging hairs. Stipules are oblong-ovate, 1-3 cm long. Leaves are elliptic, ovate in outline, with base heart-shaped or flat, margin usually 3, 5, or 7-lobed or, rarely, regularly toothed or sometimes double-toothed at leaf base. Male inflorescences are cyme-like racemes or like panicles, 5-11 cm. Female ones are in distal axils of stem, 10-28 cm, 2.5-3 mm in diameter. There are a few subspecies with differing inflorescence and leaves. **Medicinal uses:** However, the plant itself has medicinal value, and Nettle Tea has been used in Europe for many centuries. The leaves should not be touched with bare hands, but dried or boiled thoroughly in water, are used as diuretic, anti-rheumatic, anti-allergic and also for lactating mothers. The other parts of the plant are also useful for production of oils, biomass and fibre or paper. It is used in treatment of disease i.e. pimple, boils. Paste prepared from the fresh roots of alla (*Girardinia diversifolia Fries*) is applied on the pimples and boils (Tiwari and Pande 2006).
Plate 1.5 GIRARDINIA HETEROPHYLLA
Colocasia esculenta L.Schott.
It belongs to family Araceae. It is commonly known as Gadpaper/Arbi. Internanally it is called as “taro”. Leaves, stem and tubers are used as vegetable. The plant cultivated throughout the hotter parts of India. Its a tuberous perennial with a group of underground farinaceous corms consisting of a central large one and surrounding ones of varying sizes; leaves with sheathing leaf base and erect petiole upto 1.2 m long bearing a thinly coriaceous peltate –ovate, cordate lamina; spadix shorter than the petiole and much shorter than the spathe, appendix much shorter than the inflorescence. The leaves and corms are usually used as a vegetable.

The leaf juice is styptic, stimulant and rubefacient, and is useful in internal haemorrhages, otalgia, otorrhoea, adenitis and buboes. The juice of the corm is laxative, demulcent and anodyne, and useful in somatalgia, alopecia areata, haemorrhoids and congestion of the portal system (Warrier 1994). It is used in treatment of dysentery. Dried leaves of pindalu (C.esculenta Schott.) are given with salt to cure dysentery (Tiwari and Pande 2006).

The pressed juice of the petioles is styptic, and may be used to arrest arterial haemorrhage. It is sometimes used in earache and also as an external stimulant and rubefacient. The juice expressed from the leaf stalks is used with salt as an absorbent in cases of inflamed glands and buboes. The juice of the corn is used in cases of alopecia. Internally, it acts as a laxative, and is used in cases of piles and congestion of the portal system, also an antidote to the strings of wasps and other insects (Govil 1998).

It is a good source of provitamin A and Vit. C (leaves, petioles); carotene, vitB₁,vit C; good source of palmitic acid and linoleic acid (Wong et al., 1998). The leaves are good sources of alpha- tocopherol, flavonoids and amino acids (Ching and Mohamed 2001, Miean and Mohamed 2001). Taro flour is low in fat, protein and ash, but rich in starch and total dietary fiber (Tagodo species and Nip 1994). Its potential as source for the manufacture of starch, glucose syrup, and ethanol has been described extensively.
Plate 1.6 COLOCASIA ESCULENTA
Table 1.1: List of Studied Wild Edible Plants of Uttarakhand

<table>
<thead>
<tr>
<th>S.No</th>
<th>Botanical Names</th>
<th>Local Name</th>
<th>Family</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Girardinia heterophylla/diversifolia</em></td>
<td>Bichhu ghas/Kandali</td>
<td>Urticaceae</td>
<td>Srinagar Garhwal Himalaya, India,NDBR</td>
</tr>
<tr>
<td>2.</td>
<td><em>Colocasia esculenta (L.) Schott</em></td>
<td>Arbi/taro</td>
<td>Araceae</td>
<td>Kumaun</td>
</tr>
<tr>
<td>3.</td>
<td><em>Paspalum scrobiculatum</em></td>
<td>Kodo (millet)</td>
<td>Juglandaceae/Poaceae</td>
<td>Pauri Garhwal/Kumaun</td>
</tr>
<tr>
<td>4.</td>
<td><em>Rumex nepalensis Spreng.</em></td>
<td>Jangli Paalak/Payoom</td>
<td>Chenopodiaceae</td>
<td>NDBR, Uttarakhand</td>
</tr>
<tr>
<td>5.</td>
<td><em>Diplazium esculentum (Retz.)</em></td>
<td>Lingra</td>
<td>Dryopteridaceae/Athyriaceae</td>
<td>Kumaun Himal</td>
</tr>
</tbody>
</table>

2.14. ANIMAL MODEL FOR STUDY
(ALBINO MICE)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Animalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Chordata</td>
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<tr>
<td>Class</td>
<td>Mammalia</td>
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<tr>
<td>Order</td>
<td>Rodentia</td>
</tr>
<tr>
<td>Family</td>
<td>Muridae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Murinae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Mus</em></td>
</tr>
<tr>
<td>Subgenus</td>
<td><em>Mus</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>M. musculus</em></td>
</tr>
</tbody>
</table>

Our studied model is Wistar Albino Mice belongs to genus Mus and species musculus.