CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Food is essential not only for the sustenance of life, but also for the maintenance of good health. However contaminated or adulterated food is a major source of human illness. Thus quality and safety of food assumes great significance in the promotion of optimum health. Toddy is a social and local alcoholic drink produced and consumed throughout Asia, particularly India, Sri Lanka and Bangladesh. It is the partially fermented sap of coconut palm (*Cocos nucifera*), palmyra palm (*Borassus flabellifer*) and toddy palm (*Caryota urens*) or of any kind of palm. It is the undistilled alcoholic beverage and is collected through the tapping of unopened inflorescence.

Kerala maintains the highest liquor consumption in India at present. According to Economic Review the per capita consumption in the state is 8.3 litres compared to the national average of 4.1 litres. Toddy is the drink of the working class and it is considered as one of the safest nutritious drinks available. But not even 25 percent of the toddy consumed in Kerala is tapped from the trees. A simple statistics indicate that, out of more than 35 lakh litres of toddy consumed in Kerala per day, only less than 10 lakh litres are genuine. Since no tax is imposed on toddy sold through the 5,211 licensed toddy shops in the state of Kerala, no exact statistics of demand and supply are available. However, estimates are that each shop sells an average of 500 to 700 litres of toddy a day, which means that per-day consumption ranges between 25 lakh and 35 lakh litres in the state. Due to increase in consumer demand, the shortage of coconut or palm tree, shortage of tappers since the new generation is not ready to choose this job, it has often been diluted and adulterated with intoxicating drugs to get the same degree of intoxication. The adulteration of toddy primarily involves mixing cheap country arrack primarily meant for industrial purpose and chemicals like diazepam, phenobarbitone, and chloral hydrate to give high intoxication, sedation and muscle relaxing effects to the consumers (Moller, 1999; Argyropoulos and Nutt, 1999). These drugs are generally considered to be dangerous when consumed along with
alcohol, and have proved to be fatal poisonings according to the analysis of forensic toxicological investigations conducted by Koski et al., 2002.

The adulteration of toddy was increased in the state of Kerala ever since the sale of arrack was banned from 1st April 1996. Most of the arrack drinkers have turned to toddy. Habitual drinkers prefer toddy with high intoxication to normal toddy as they do not get the intoxicating effect from normal toddy which they get from arrack, adulteration of toddy became a common practice. Consumption of spurious toddy leads to chronic health disorders in humans include hepatic problems like fatty liver, fibrosis leading to cirrhosis, improper functioning of kidneys, prolonged sedation to brain. Cases of many hooch tragedies have been reported from various parts of Kerala. Casualties leading to human fatalities have occurred. Every day, hundreds of people fall prey to the flourishing illegal liquor trade in our country. According to official figures, 225 people have died in Kerala because of hooch in the last 20 years. The unofficial figure is said to be much higher. The following are some of the major poisoning cases reported in the state of Kerala.

The 1982 Vypeen (near Kochi) liquor tragedy accounted for the highest death toll of 78, followed by Punalur (34) in 1981 and Kollam-Kalluvathukkal (32) in 2000 (Source: Dept. of Excise, Govt. of Kerala). Seventeen people, including a woman, lost their life and a dozen others hospitalized after consuming poisoned toddy at Malappuram district on September 2010. But very little information is available about the characteristics of natural toddy in detail. So the present study was undertaken, by incorporating a comparison of natural and adulterated toddy and to evaluate the toxicological effects of the three common adulterants, i.e. diazepam, phenobarbitone and chloral hydrate.

**OBJECTIVES OF THE STUDY**

- To study the physical and chemical properties of natural toddy viz, turbidity, odour, percentage transmission of supernatant, pH, alcohol content, ascorbic acid, phosphorous, potassium, sodium, total acidity, ash content and alkalinity of water soluble ash.
To study the ingredients in adulterated toddy viz, silicates, starch, sodium lauryl sulphate, saccharin, diazepam, phenobarbitone and chloral hydrate.

To study the toxicological effects of natural toddy, shop toddy, diazepam, phenobarbitone and chloral hydrate alone and in combinations with toddy on experimental animals.

To study the toxicological effects of natural toddy, shop toddy, diazepam, phenobarbitone and chloral hydrate alone and in combinations with toddy on normal chang liver cells.

REVIEW OF RELATED LITERATURE

1.1 PRODUCTION OF TODDY

There are a number of genera of palm that are prevailing, which include coconut palm (*Cocos nucifera*), palmyra palm (*Borassus flabellifer*), toddy palm (*Caryota urens*), date palm (*Phoenix dactylifera*), nipa palm (*Nypa fruticans*) (Stanton and Owen, 2002).

In south India, especially in Kerala, coconut palm is used for tapping toddy. The word tapping connotes the extraction and various manipulations for stimulating the different toddy yielding palms to exude juice from a selected part. The methods of tapping the coconut palm are very ancient. The tapping process can be summarized as follows. When the palm has reached the normal bearing stage, every leaf axil produces a spadix or inflorescence. The young inflorescence is tightly bound with twigs and beaten with a weighted wooden mallet or animal bone during morning and evening, for a number of days. When the inflorescence begins to ooze its sap, the tip is cut and the sap allowed trickling into an earthenware pot (Browning and Simmons, 1916). Owing to the yeasts and other organisms already present in the used pots, alcoholic fermentation begins immediately. The tapper usually changes the pot twice daily. Each morning and evening a tapper climbs the tree to collect the toddy, and at each visit he shaves off a fine transverse section of the inflorescence so as to leave a new oozing surface. The fermented toddy, which is milky in appearance, is brought to various licensed...
toddy shops for sale within a few hours of collection (Grimwood and Ashman, 1975).

1.2 CHEMICAL AND MICROBIAL COMPOSITION OF TODDY

Studies showed that the toddy undergo various biochemical as well as microbial changes during the natural fermentation. The palm sap fermentation involved alcoholic-lactic-acetic acid fermentation, by the presence of mainly yeasts and lactic acid bacteria. Aidoo et al., 2006 concluded that *Saccharomyces* spp. present in the natural fermented palm sap and are important for the formation of characteristic aroma of the palm wine. The major microorganisms responsible for the fermentation of the wild date palm sap include *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been reported to be the dominant yeast species (Odunfa and Oyewole, 1998). Other yeast species such as *Acetobacter aceti*, *A. rancens*, *A. suboxydans*, *Leuconosticdextranicum*, *Micrococcus* sp., *Pediococcus* sp., *Bacillus* sp., *Sarcinas*p (Shamala and Srikantiah, 1988) are also present. The microorganisms are reported to originate from the palm tree, the gourd used for sap collection and fermentation, or the tapping equipment. Study done by Atputharajah et al.,( 1986) reported the presence of seventeen species of yeasts and seven genera of bacteria in the natural fermented coconut palm sap. All of these strains were evolved over the centuries, and have acquired properties that make them uniquely suited for the purpose of converting sugars in the juice or extract to ethanol and CO₂.

Fresh toddy is sweet to taste, oyster white and translucent fluid with the pH ranging from 6.2 to 7.2. It is an excellent beverage and a rich source of sugar. It is also a good source of baker’s yeast. Sucrose is its main constituent. The average constitution of fresh toddy is given below(Browning and Simmons, 1916).

<table>
<thead>
<tr>
<th>Table 1.1 Major constituents of toddy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total solids</strong></td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
</tr>
<tr>
<td><strong>Ash</strong></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
</tr>
<tr>
<td><strong>Ascorbic acid</strong></td>
</tr>
</tbody>
</table>
There is a widespread belief that coconut toddy is a rich source of the B-complex vitamins on account of the yeast present. The vitamin B1 content of coconut toddy was reported as < 5 I.U/100g. Half of the total sugars are fermented during first 24 hours and ethanol content of the fermented palm sap reaches maximum of 5.0-5.28 % (v/v) after 48 hours (Sekar and Mariappan, 2005). Atputharajah et al.,(1986) studied the chemical changes of coconut palm sap during fermentation and reported that ethanol content of naturally fermented coconut palm sap reached maximum(approximately 9% v/v) after five days fermentation. A constant pH drop was observed at the initial fermentation stage, and sugar conversion begins and produces ethanol at constant pH. Shamala and Srikantiah (1988) reported that the fermentation produces mainly ethanol, acetic acid and lactic acid. pH of the sap rapidly dropped from around 7.2 to 5.5 due to formation of acetic acid and ethanol content drastically increased to 5%(v/v) within 8 hours. Toddy contains the following %/volume of ethyl alcohol.

**Table 1.2. Maximum ethyl alcohol percentage in toddy as fixed by Kerala Abkari Shops (Disposal) Rules, 2002**

<table>
<thead>
<tr>
<th>Nature of tree</th>
<th>%/V of ethyl alcohol(Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut toddy</td>
<td>8.1</td>
</tr>
<tr>
<td>Palmyra toddy</td>
<td>5.2</td>
</tr>
<tr>
<td>Choondapana toddy</td>
<td>5.9</td>
</tr>
</tbody>
</table>


Rule 9(2) of the Kerala Abkari Shops (Disposal) Rules, 2002 says that ethyl alcohol content in toddy on sale should not exceed the above values.

Studies at the Sri Lanka Coconut Research Institute in 1957 reported that toddy collected at 7 a.m, using unsterilized earthen pots already contains 2.9 % (w/w) of alcohol and 9.58% of fermentable sugars. Fermentation continued and alcoholic content reached a maximum of 8.2% after 45 hour, however 8.1 % had been reached after 33 hour. When sterilized pots were used, alcohol was not detected, fermentation was set in slowly and only became rapid after 9 hours, a maximum of alcohol was reached after 60 hour 6.1% but 5.7% had been reached.
after 36 hour. At the maximum alcohol stage there still remains some fermentable sugars, but on prolonged standing alcohol was converted into acetic acid. They found an acidity range of 0.32-0.67 % and alcohol content 2.7- 5.8 % (w/w) (Child, 1972). It has been reported that fresh toddy contains most of vitamins, especially thiamine (0.3mg/100gm), riboflavin (2.7mg/100gm), nicotinic acid (218ug), ascorbic acid (16-30mg) etc per 100 ml of toddy and the content may change during fermentation. Leong, 1953 reported the major inorganic constituents of partially fermented toddy. The composition and quality of palm sap are greatly affected by the location, weather, time and duration of tapping (Borse et al., 2007).

| Nitrogen                        | 0.033- 0.038g/100ml |
| Phosphoric acid (as P₂O₅)      | 0.015-0.023g/100ml  |
| Potassium(as K₂O)              | 0.144-0.203g/100ml  |
| Calcium (as CaO)               | 0.0017-0.0021g/100ml|
| Magnesium (as MgO)             | 0.006-0.0085g/100ml |
| Manganese (as Mn)              | 44-60 µg/100ml      |


According to Indian Standard for alcoholic drinks-toddy specification, there were certain requirements prescribed. Toddy shall be free from added coloring and foreign materials and shall also be free from any ingredient injurious to health. Toddy shall also comply with the requirements given in the table below.
Table 1.4. Requirements for toddy

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Characteristics</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total acid as tartaric acid (expressed in terms of 100 litres of absolute alcohol) Max</td>
<td>400g</td>
</tr>
<tr>
<td>2</td>
<td>Volatile acid as acetic acid (expressed in terms of 100 litres of absolute alcohol) Max</td>
<td>100g</td>
</tr>
<tr>
<td>3</td>
<td>Copper, Max</td>
<td>10mg/kg</td>
</tr>
</tbody>
</table>


As per the above IS toddy shall be free from any ingredient injurious to health. As per the Kerala Abkari Shops Disposal(Amendment)Rules 2007, toddy means “fermented juice drawn from any coconut, palmyra or choondapana palm and conforming to such specifications and restrictions as may be notified by the Government based on scientific studies and Indian Standard Specifications”

1.3 ABKARI ACT IN KERALA

Public well-being is the foremost aim of prohibition laws. The main purpose of the Abkari Act is to bring about social harmony by curbing excessive consumption of alcohol and thus make possible the realization of the constitutional goal enunciated in Article 47 of the Constitution of India.

According to Arthasastra of Kautilya, the ill-fated effects of drinking are loss of money, lunacy in a sensate man, corpse like appearance while living, nakedness, the loss of knowledge of Vedas, loss of life, wealth, and friends, disassociation with the good, suffering from pain, and indulgence in playing on musical instruments and in singing at the expense of wealth. Manusmriti condemns drinking. Drunkenness is considered as a vice and most of the known religious traditions abhor it. Islam for centuries has prohibited even the moderate use of the fermented drink. Spirituous drinks were popular in Bengal during the Mughal era as a high demand item and it was always an important source of Government revenue. The Mughal Government had an Abkari Duftar to regulate production of spirituous liquors and collection of tax from manufactures and dealers. It became the Abkari department during the British East India Company period and the Customs and Excise Department subsequently.
The production and distribution of liquors was a monopoly of the state ever since 1773. The main reason was partly for revenue and partly for cultural reasons of the colonial Government’s conscious policy to encourage the consumption of alcohol. Consequently the consumption of liquors increased so much that in the nineteenth century and every village market had a corner for the sale of spirits. Both Hindu and Muslim reformists demanded the total abolition of alcohol. In response to such popular reaction, the government adopted the policy of restricting the production, import of abkari and raising taxation.

In 1790, East-India Company introduced Abkari Excise system in India. The first legislation in British India on Abkari Revenue is the Madras Act XIX of 1852. Unabated consumption of liquor was on the rise and it alarmed all those concerned with public health and morals. Excise policy was reconsidered after many strong demands. In 1937, for the first time, popular Ministries introduced prohibition of intoxicating drink or drug in parts of Madras, Bombay, Uttar Pradesh, Bihar and the Central Provinces. The Prohibition Act 1950 regulated prohibition. It however did not achieve the intended object. Therefore, by Notification issued as SRO. No.104/67, Government suspended the operation of the provisions of the Prohibition Act and revived the provisions of Abkari Act. The Act was passed by His Highness the Maharaja of Cochin on the 5th day of August 1902 and extended to the whole of Kerala as per Act 10 of 1967, which received the assent of His Excellency the President of India on 29th July, 1967.

Section 57A of the Abkari act (Abkari Act 1 of 1077)
This section is relevant in the present study

Section57A. For adulteration of liquor or intoxicating drug with noxious substances etc

(1) Whoever mixes or permits to be mixed any noxious substance or any substance which is likely to endanger human life or to cause grievous hurt to human beings, with any liquor or intoxicating drug shall, on conviction, be punishable-

(i) if, as a result of such act, grievous hurt is caused to any person, with imprisonment for a term which shall not be less than two years but
which may extend to imprisonment for life, and with fine which may extend to fifty thousand rupees;

(ii) if, as a result of such act, death is caused to any person, with death or imprisonment for a term which shall not be less than three years but which may extend to imprisonment for life, and with fine which may extend to fifty thousand rupees;

(iii) in any other case, with imprisonment for a term which shall not be less than one year, but which may extend to ten years, and with fine which may extend to twenty-five thousand rupees.

1.4 SURVEY THROUGH DEPARTMENT OF EXCISE, GOVT. OF KERALA

In Kerala there were 5972 licensed toddy shops before the ban of arrack in 1996. But according to the Abkari Policy of 2002-2003, 33% toddy shops were abolished and in the same year 356 shops were given license. Accordingly, the total number toddy shops became 4356 during the year 2002-2003. During 2003-2004, 6 toddy shops were reinstated and the shop number again increased to 4362. On account of the lack of labour of abkari workers and the increase in the number of labours, the Govt of Kerala in the Abkari Policy of 2007-08, reinstated 1610 toddy shops and toddy shop number hiked to 5972. According to the Abkari Policy of 2008-09, shops which were not functioning in the previous financial year and the shops which were reinstated in Thiruvananthapuram, Kollam and Pathanamthitta, were stopped functioning and reinstated one shop in Vaikom Excise Range, and cancelled the license of 4 other shops. The net result shows the number of licensed toddy shops were 5211. During the next four years the number of licensed toddy shops has come down to 4765.

Trading network of Abkari system
(Source: Dept of Excise, Govt of Kerala)

According to entry 8 of list 2 of schedule 7 of the Constitution of India, intoxicating liquors, that is to say, the production, manufacture, possession, transport, purchase and sale of intoxicating liquors is a matter of legislation by the states. Similarly levy of duty on all alcoholic liquors fit for consumption is also a
matter of legislation by the state under entry 51 of the same list (The Kerala State Excise Manual Vol II, 1972, p.5)

![Diagram of Abkari network]

**The Excise revenue from toddy is derived from the following ways**

1. License fee which is assessed on toddy shops.
2. Taxes imposed on the tapping of toddy trees.

   The tree tax is assessed for two halves in a year. For coconut trees the tax is assessed for six months and it is Rs.30/tree. For Palmyra palm, it is assessed for one year and is Rs.100/tree, for sago palm it is for six months and is Rs.50/tree.
Table 1.5 The revenue collected through toddy shop rental

<table>
<thead>
<tr>
<th>YEAR</th>
<th>AMOUNT (Crores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996-1997</td>
<td>57.22</td>
</tr>
<tr>
<td>1997-1998</td>
<td>124.78</td>
</tr>
<tr>
<td>1998-1999</td>
<td>83.37</td>
</tr>
<tr>
<td>1999-2000</td>
<td>124.25</td>
</tr>
<tr>
<td>2000-2001</td>
<td>146.44</td>
</tr>
<tr>
<td>2001-2002</td>
<td>23.57</td>
</tr>
<tr>
<td>2002-2003</td>
<td>23.60</td>
</tr>
<tr>
<td>2003-2004</td>
<td>14.15</td>
</tr>
<tr>
<td>2004-2005</td>
<td>20.48</td>
</tr>
<tr>
<td>2005-2006</td>
<td>22.8</td>
</tr>
<tr>
<td>2006-2007</td>
<td>35.71</td>
</tr>
<tr>
<td>2007-2008</td>
<td>36.66</td>
</tr>
<tr>
<td>2008-2009</td>
<td>25.98</td>
</tr>
<tr>
<td>2009-2010</td>
<td>24.00</td>
</tr>
<tr>
<td>2010-2011</td>
<td>20.20</td>
</tr>
<tr>
<td>2011-2012</td>
<td>20.71</td>
</tr>
<tr>
<td>2012-2013</td>
<td>19.8</td>
</tr>
<tr>
<td>2013-2014</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Source: Govt. of Kerala, Taxes Dept (A&G), Thiruvananthapuram.

In the year of 1996, the Govt. of Kerala prohibited preparation, possession and sale of arrack. During the next few years the revenue collected through toddy shop rental had increased tremendously.

Table 1.6 The List of trees licensed in the state of Kerala

<table>
<thead>
<tr>
<th>Year</th>
<th>Coconut</th>
<th>Palmyrah</th>
<th>Sago</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-2010</td>
<td>582995</td>
<td>39735</td>
<td>32844</td>
</tr>
<tr>
<td>2010-2011</td>
<td>448168</td>
<td>53778</td>
<td>45143</td>
</tr>
<tr>
<td>2011-2012</td>
<td>920384</td>
<td>24289</td>
<td>32139</td>
</tr>
<tr>
<td>2012-2013</td>
<td>853542</td>
<td>21168</td>
<td>30260</td>
</tr>
<tr>
<td>2013-2014</td>
<td>816573</td>
<td>23659</td>
<td>31715</td>
</tr>
</tbody>
</table>

Source: Board of Revenue- Dept. of Excise, Govt. of Kerala
Table 1.7 Average production of toddy/tree/day

<table>
<thead>
<tr>
<th></th>
<th>Production (litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut toddy</td>
<td>1.5 litres</td>
</tr>
<tr>
<td>Palmyra</td>
<td>4.5 litres</td>
</tr>
<tr>
<td>Choondapana</td>
<td>6.75 litres</td>
</tr>
</tbody>
</table>

Source: Board of Revenue- Dept. of Excise, Govt. of Kerala

Table 1.8 Toddy production and consumption during 2013-14

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>District</th>
<th>Production (litres)</th>
<th>Consumption(litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thiruvananthapuram</td>
<td>100800</td>
<td>100800</td>
</tr>
<tr>
<td>2</td>
<td>Kollam</td>
<td>346870</td>
<td>1009095</td>
</tr>
<tr>
<td>3</td>
<td>Pathanamthitta</td>
<td>2781375</td>
<td>5516098</td>
</tr>
<tr>
<td>4</td>
<td>Alappuzha</td>
<td>14663125</td>
<td>30249775</td>
</tr>
<tr>
<td>5</td>
<td>Kottayam</td>
<td>21327712</td>
<td>34186321</td>
</tr>
<tr>
<td>6</td>
<td>Idukki</td>
<td>3018740</td>
<td>5218736</td>
</tr>
<tr>
<td>7</td>
<td>Ernakulam</td>
<td>94545220</td>
<td>109875720</td>
</tr>
<tr>
<td>8</td>
<td>Thrissur</td>
<td>28132192</td>
<td>34479177</td>
</tr>
<tr>
<td>9</td>
<td>Palakkad</td>
<td>116205570</td>
<td>36054090</td>
</tr>
<tr>
<td>10</td>
<td>Malappuram</td>
<td>3908627</td>
<td>4880158</td>
</tr>
<tr>
<td>11</td>
<td>Kozhikode</td>
<td>8353584</td>
<td>11043584</td>
</tr>
<tr>
<td>12</td>
<td>Wayanad</td>
<td>1769175</td>
<td>2515950</td>
</tr>
<tr>
<td>13</td>
<td>Kannur</td>
<td>15068747</td>
<td>15068747</td>
</tr>
<tr>
<td>14</td>
<td>Kasargode</td>
<td>5390220</td>
<td>6201120</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>315611957</strong></td>
<td><strong>296399371</strong></td>
</tr>
</tbody>
</table>

Source: Board of Revenue- Dept. of Excise, Govt. of Kerala

The Excise Department claims Kerala produces around 8.35 lakh litres of toddy a day and consumes 7.21 lakh litres. Even though the official figures tally with the production and consumption, the actual facts are contradictory. There is a general allegation that Kerala consumes more toddy than it produces. By a rough estimate, the State consumes about two lakh more litres of toddy than it actually
produces, making spurious toddy sales worth more than Rs. One crore a day at the price of Rs.60 /litre.

Adulterants like diazepam, phenobarbitone, chloral hydrate, Chlorpheneramine maleate (CPM), an active content in antihistamines etc are also used which are highly injurious to health of toddy consuming common people. In the state of Tamilnadu toddy tapping is banned. One argument in favour of ban on toddy tapping was that of adulteration. The sap could easily be adulterated to add to intoxication. According to the Prevention of Food Adulteration Act and the Abkari Act toddy should be free from harmful ingredients injurious to health. Despite this, adulterated toddy is very often reported by state Excise department. Such toddy samples are very often submitted to the Chemical Examiner’s laboratory or Forensic Science Laboratory under the state Government for the analysis of their alcohol content and detection of adulterants.

1.5 CNS DEPRESSANTS (Rossi, 1963).

Central nervous system depression or CNS depression refers to physiological depression of the central nervous system that can result in decreased rate of breathing, decreased heart rate, and loss of consciousness possibly leading to coma or death. CNS depression often results from the use of depressant drugs such as alcohol, opioids, barbiturates, benzodiazepines, general anesthetics, and anticonvulsants such as valproate semisodium used to treat epilepsy. Drug overdose is often caused by combining two or more depressant drugs, eventhough overdose is certainly possible by consuming a large dose of one depressant drug. CNS depression can also be caused by the accidental or intentional inhaling of certain volatile chemicals such as Butanone (contained in Plastic Cement). Depressants exert their effects through a number of different pharmacological mechanisms, the most prominent of which include facilitation of GABA or opioid activity, and inhibition of glutamatergic or catecholaminergic activity.

1.6 BENZODIAZEPINES

The commonly used adulterant, diazepam comes under a class of medicines called Benzodiazepines. Benzodiazepines are used for their properties as anesthetics,
antidepressive, hypnotic, tranquilizers and sedatives. Benzodiazepines are psychotropic drugs widely used in the treatment of anxiety and insomnia (Rickles et al., 2000). In addition to their therapeutic use, they are susceptible to abuse because of their additive properties. In postmortem investigations, alcohol is frequently detected both alone and in combination with drugs. In the 1980s, it was reported that presence of alcohol reduces the blood concentration at which a given proportion of poisonings by certain drugs occurs (King, 1982; Stead and Moffat, 1983; Poikolainen, 1984). Benzodiazepine drugs are generally considered relatively safe when taken alone but dangerous in combination with alcohol (Gaudreault et al., 1991; Hobbs et al., 1996; Dollery, 1999). Data from postmortem alcohol and drug determinations in Finland between 1995 and 2000 were analyzed statistically to evaluate as to which drugs act synergistically with alcohol in drug-alcohol fatalities. This study investigated the relationship between the blood alcohol concentration and the presence of some benzodiazepines, in fatal poisoning (Vuori et al., 2001).

In addition to the anxiety disorders, benzodiazepines have utility in other diseases both psychiatric (Pollack, 1993) and neurological. Their use in epilepsy is well established (Sato, 1989). The administration of benzodiazepines during alcohol withdrawal is crucial, especially in relation to the potential development of seizures and delirium tremens. Their safe profile and sedative effect makes them extremely useful in agitated states (Anton and Becker, 1995).

**Advantages and disadvantages**

Perhaps the major reason why the benzodiazepines were welcomed so potentially when they first became available was their safety. In contrast to the barbiturates and meprobamate, the benzodiazepines are safe in overdose, almost never leading to fatalities when taken alone in people without concurrent medical illness.

In addition, they have a number of positive attributes. They work quickly, often producing symptoms of relief within minutes. Therapeutic benefit tends to increase over several weeks and, in general, therapeutic gains are maintained for weeks or months, without significant tolerance or escalation of dose developing in most cases, even after many years of use (Rickles and Schweizer, 1986).
However, tolerance does develop to some of the unwanted actions of the benzodiazepines, such as sedation, ataxia and memory impairment, although this may never be complete. The only area in which tolerance develops to the therapeutic actions of benzodiazepines is epilepsy, where breakthrough seizures can occur after weeks or months. The other main drawback of benzodiazepines is their abuse potential. There is also concern that some benzodiazepines increase the risk for road traffic, and possibly other, accident, especially when used concurrently with alcohol. Combination of alcohol and diazepam elicits additive or supra-additive effects (Vapaatalo and Karppanen, 1969; Linnoila and Mattila, 1973a; Linnoila and Hakkinen, 1974; Mehar et al., 1974; Morland et al., 1974). The basis for these additive or supra-additive effects of ethanol and diazepam in man has not been established.

However, in a recent study it was found that ethanol elevated plasma and brain levels of diazepam by approximately 6-fold in the rat. When extrapolating these data to man, one is immediately confronted with the fact that the major route of metabolism in the rat is hydroxylation of the 5-phenyl ring, a pathway nonexistent or of minor significance in man (Whitehouse et al., 1975).

The effect of ethanol (3 g/kg, orally), administered 0.5 h before [C\textsuperscript{14}]-diazepam (5 mg/kg, orally), on the pharmacokinetics of diazepam in male Swiss Webster mice was examined. At 4, 6, 8 and 12 h after dosing, blood levels of [C\textsuperscript{14}]- were higher in ethanol pretreated mice than in vehicle pretreated controls. Ethanol reduced oxazepam levels but increased desmethyl diazepam levels, suggesting that 3-hydroxylation of desmethyl diazepam were inhibited by ethanol (Paul and Whitehouse, 1977).

Ethanol known to inhibit the drug metabolizing enzyme system has been reported previously in man thus preventing the elimination of lipid soluble material from the body (Rubin and Lieber, 1968; Rubin et al., 1970; Coldwell et al., 1973; Mezey, 1976).
Synergism by ethanol with the effect of diazepam on the central nervous system is one possible explanation for the supra-additive or potentiating effects of the ethanol-diazepam combination (Paul and Whitehouse, 1977).

Benzodiazepines can occasionally induce cardiovascular and pulmonary toxicity. There is clear evidence that the prolonged use of even therapeutic doses of benzodiazepines will lead to dependence. The risk of developing significant withdrawal symptoms is related to dosage and duration of treatment. Prevention of gastrointestinal absorption should be initiated in all intentional benzodiazepine overdoses (Gaudreault et al., 1991).

1.7 BARBITURATES

Phenobarbitone, one of the toddy adulterants detected comes under the class of drugs called barbiturates.

**Pharmacology:** (Ito et al., 1996)

Barbiturates are nonselective central nervous system (CNS) depressants, capable of producing all degrees of depression from mild sedation and hypnosis to general anesthesia, deep coma and death. The extent of CNS depression varies with the route of administration, dose and pharmacokinetic characteristics of the particular barbiturate. Patient specific factors such as age, physical or emotional state and the concomitant use of other drugs will also affect response. The mechanism of action of barbiturates is not completely known. They may act by enhancing and/or mimicking the synaptic action of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. The sedative-hypnotic action of barbiturates may be due to an inhibition of conduction in the reticular formation resulting in a decrease in the number of impulses reaching the cerebral cortex.

Anticonvulsant activity may result from a reduction in CNS synaptic transmission and an increase in the threshold for electrical stimulation of the motor cortex. Phenobarbital is the only barbiturate with anticonvulsant activity at sub hypnotic doses.

**Pharmacokinetics:** After oral administration, absorption is usually rapid and relatively complete. The sodium salts undergo rapid dissolution and are absorbed
more quickly than their corresponding free acids. The rate of absorption is increased when the barbiturate is formulated as a liquid, when the stomach is empty and when alcohol is ingested concurrently. Once absorbed the barbiturates are rapidly distributed to all tissues and fluids. High concentrations appear in the brain, liver and kidneys.

**Adverse Effects** (Schlatter et al., 2001)

Drowsiness is frequent especially at initiation of therapy and may persist throughout the next day after hypnotic doses. Mild impairment of concentration, judgement, memory, and fine motor skills may occur. Disturbances of sleep, dizziness, vertigo, headache and depression may occur. Patients with uncontrolled pain may experience paradoxical euphoria, elation, excitement and confusion. In children, hyperactivity is not uncommon; behavioural disturbances and cognitive impairment may occur. Geriatric patients may experience excitation, confusion or depression.

Cardiovascular: Hypotension may be observed with i.v. administration and is generally related to the rate of administration.

Respiratory: Respiratory depression.

Hypersensitivity: Facial edema, skin rash (1 to 2%) may be purpuric, vesicular or erythematous. Exfoliative dermatitis and erythema multiforme are rare. Hypersensitivity reactions have a greater tendency to occur in patients with a history of asthma, urticaria or angioedema.

Hepatic: Severe allergic reactions may result in jaundice due to degenerative changes in the liver. Toxic hepatitis is rare.

Hematologic: megaloblastic anemia (responds to folic acid therapy). Agranulocytosis and thrombocytopenia are rare.

Metabolic: Barbiturates may increase vitamin D requirements, possibly by increasing vitamin D metabolism via enzyme induction. Rarely, rickets and osteomalacia have been reported following prolonged use of barbiturates. Gastrointestinal: nausea, vomiting, diarrhea and constipation.
Miscellaneous: Exacerbation of porphyria, pain at the injection site.

Barbiturates are medicines that act on the central nervous system and cause drowsiness and can control seizures. Also known as sedative-hypnotic drugs, barbiturates make people very relaxed, calm, and sleepy which depress or slow down the body's functions. These drugs are sometimes used to help patients relax before surgery.

Since alcohol is also a CNS (central nervous system) depressant, the effects due to using barbiturates in conjunction with alcohol are multiplied and the risk of death increases. Overdose deaths are more frequent when alcohol and barbiturates are mixed, whether accidentally or deliberately.

Barbiturates are also a popular “street” drug. Commonly abused barbiturates include amobarbital (Amytal), pentobarbital (Nembutal), and secobarbital (Seconal). These drugs account for approximately one-third of all reported drug-related deaths, including suicides and accidental drug poisonings. Accidental deaths may occur when a user takes one dose, becomes confused, and unintentionally takes an additional or larger dose.

**Physical Effects**

The effects of barbiturates are much like the effects of alcohol. Small amounts produce calmness and relax muscles. Larger doses cause slurred speech, staggering, and poor judgement. High doses can cause unconsciousness and death. Effects of prescribed doses of short-acting barbiturates such as secobarbital generally last 4 - 6 hours while effects from phenobarbital, a longer-acting barbiturate will last from 8 - 12 hours. When taken, barbiturates slow down CNS activities such as heart beat, breathing, brain activities and reflexes.

**Paired with Alcohol**

Taking barbiturates with alcohol is extremely risky, as it severely increases the probability of death. Alcohol increases the depressant power of the drugs, as the liver processes the alcohol first – leaving the barbiturates to saturate the organ system for a longer duration of time. Barbiturates and alcohol are
frequently found in combination in cases of accidental or intentional fatal self-poisoning. With alcohol, even a small amount of barbiturates can cause death (Stead and Moffat, 1983).

Phenobarbitone is a usually detected adulterant in toddy. Symptoms of phenobarbital dependence are similar to those of chronic alcoholism. In the presence of alcohol the lethal dose of barbiturate is far less.

**Action of ethanol on the metabolism of barbiturates or ethanol and Cytochrome p-450**

Ethanol inhibits the metabolism of barbiturates by inhibiting hydroxylation of barbiturates by the hepatic endoplasmic reticulum. When barbiturates are metabolized, the reaction is catalyzed by NADPH-dependant cytochrome system, specifically cytochrome p-450.

Cytochrome p-450 is similar to other cytochromes because it is able to bind to oxygen and carbon monoxide. Cytochrome p-450 is found in the endoplasmic reticulum of the liver and is responsible for hydroxylating compounds (barbiturates). The production of cytochrome p-450 is stimulated by the presence of barbiturates. By hydroxylating the barbiturates, derivations of the barbiturate are more water soluble and can be removed through the blood, filtered by the kidney, and excreted through the urine. The reaction is as follows:

\[
\text{RH} + \text{NADPH} + \text{Hydronium ion} + \text{Oxygen} \rightarrow \text{R-OH} + \text{NADP}^+ + \text{Water}
\]

Any unmetabolized barbiturates will be excreted through the urine. Therefore cytochrome p-450 is responsible for detoxification, metabolism, and excretion (Matthews and van Holde, Biochemistry)

The most dangerous thing to mix with a sedative (alcohol) is another sedative or “downer”. The combination can lead to extreme depression of the Central Nervous System and can be fatal. When combined with alcohol these drugs have a synergistic effect, meaning that the combined depression of the CNS is greater than the sum of the depression caused by alcohol and that of the sedative. This effect can be expressed with the equation 1+1=3 (the combined effect is more intense than the separate effects combined).
Central Nervous System

Alcohol has the effect of inhibiting the hydroxylation of barbiturates, which means that in the presence of alcohol, barbiturates remain at high levels since they are not being metabolized and CNS depression is increased. Chronic alcohol consumption causes changes in the sensitivity of CNS to barbiturates. As the tolerance for alcohol occurs a tolerance to barbiturates develops, also referred to as cross tolerance (one drug affects another drug). Sober alcoholics are less sensitive to barbiturates for two reasons viz; decreased CNS sensitivity and increased production of hydroxylating enzymes in the liver (Wesson. et al.,1977).

Alcohol and barbiturates are both depressants that interact with gamma-aminobutyrate (GABA)-activated chloride channel. Gamma-aminobutyric acid is a neurotransmitter that has an inhibitory effect to the response of the central nervous system. The chloride channels remain open for an increased amount of time, keeping the cell's cytoplasm negative and not allowing for it to reach threshold. Without reaching threshold, no action potential can occur, and the signal is inhibited. Barbiturates' primary site of action is the brain because of the ability to cross the blood brain barrier quickly. The liver also plays an important role in metabolizing barbiturates into readily soluble barbiturate derivatives (Eckert et al., 1988).

Psychological Effects

Barbiturates produce a feeling of euphoria, tranquility and temporary relief of anxiety. Regular and prolonged use of barbiturates induces tolerance—the need for higher doses of a drug to produce the desired effect. Physical and psychological dependence and withdrawal symptoms occur when use of the drug is abruptly stopped. Withdrawal symptoms range from restlessness, insomnia and anxiety to convulsions and death. (Devenyi and Harrison, 1985)

Because the drug can easily pass through the placenta, use of barbiturates during pregnancy may cause birth defects and behavioural problems in babies. Babies may be physically dependent on the drug at birth and experience withdrawal symptoms shortly after they are born. Their symptoms may include
breathing problems, feeding difficulties, disturbed sleep, sweating, irritability, and fever (Nau et al., 1982)

1.8 CHLORAL HYDRATE (CH)

Chloral hydrate (2,2,2-trichloro-1, 1-ethandiol) is a rapidly effective sedative and hypnotic drug that is often prescribed to infants, young children, and elderly patients prior to surgical procedure to relieve anxiety or produce sleep (Drug Information, American Hospital Formulary Service, 1993). Chloral hydrate has been used routinely as a sedative in pediatric dentistry (Frush et al., 1996). Chloral hydrate is also used in veterinary medicine as a central nervous system depressant and anesthetic (Rossoff, 1974). The general public is exposed to CH in small amounts through drinking water since it is formed as a disinfection by-product when water is treated with chlorine (National Academy of Science, 1977; Merdink et. al., 1999; National Toxicology Program, 2000).

Chloral hydrate (CH) is a commonly found disinfection by-product in water purification, a metabolite of trichloroethylene, and a sedative/hypnotic drug. Chloral hydrate and two of its reported metabolites, trichloroacetic acid (TCA) and dichloroacetic acid (DCA), are hepatocarcinogenic (Harren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991). Trichloroacetic acid has been proposed as the metabolite responsible for the carcinogenic activity due to its higher systemic concentrations and long half-life (Elcombe et al., 1985).

At the lower chloral hydrate doses, a greater fraction of the dose is converted into trichloroacetic acid. The direction of metabolism shifts towards producing more free trichloroethanol (f-TCE) than trichloroacetic acid at the higher doses. The same shift towards reductive conversion of chloral hydrate into free - trichloroethanol was reported by Watanabe and Nakamura (1998) with co administration of ethanol. A change in the redox state of the liver by the ethanol metabolism was identified as the cause. With the high dose of chloral hydrate infusion, the redox state may be influenced by the glucuronidation of trichloroethanol or by the secondary input of trichloroethanol from the enterohepatic circulation (EHC) of trichloroethanol. Chloral hydrate is rapidly
absorbed and metabolized in the liver (Lipscomb et al., 1996). LD$_{50}$ of chloral hydrate in rats is 285mg/kg (Boitsov et al., 1970).

Kaplan et al., (1967) investigated the effect of ethanol consumption on the metabolism of chloral hydrate in adults. In subjects consuming both ethanol and chloral hydrate, the concentration of trichloroethanol in blood rose more rapidly and reached a higher concentration than in subjects consuming chloral hydrate only.

In general, chloral hydrate should not be combined with any drugs that make you sleepy or otherwise depress the central nervous system. These might be alcohol, sleeping pills, anti-seizure drugs, anti-anxiety drugs, benzodiazepines, barbiturates, narcotic pain killers, illegal narcotics, muscle relaxants, antihistamines, and most cold and allergy medications. Some specific drugs that interact with chloral hydrate are arsenic, paraldehyde, diuretics, anticoagulants, cisapride, astemizole, dofelidile, astemizole, terfemadine, carisoprodol, clobazam, codeine, diazepam, Valium, pimozide, thioridazine, Ziprasidone, mesoridozine, and levomethadyl. In humans, chloral hydrate is rapidly absorbed and then either oxidized to trichloroacetic acid (8%) or reduced to trichloroethanol (92%), mainly by the liver, but also by the kidney. Trichloroethanol may be conjugated with glucuronic acid to form trichloroethanolglucuronide (TCOG; urochloralic acid), an inactive metabolite (Ogino et al., 1990; McEvoy, 1999). Additional trichloroacetic acid is formed during enterohepatic circulation of trichloroethanol, such that 35% of the initial dose of chloral hydrate is converted to trichloroacetic acid. The erythrocytes also metabolize chloral hydrate to trichloroethanol, mainly via alcohol dehydrogenase (Allen and Fisher, 1993).

**Pharmacology of Chloral Hydrate**

Chloral hydrate's pharmacological properties enhance the gamma-amino butyric acid receptor complex. It is believed that chloral hydrate has general CNS depressant effects due to its active metabolite, trichloroethanol. It accomplishes this by rapid and extensive metabolization in the liver and erythrocytes by alcohol dehydrogenase. A small amount of chloral hydrate and a larger portion of trichloroethanol are oxidized to a minor, less active metabolite, trichloroacetic
acid, in one's liver and kidneys. This metabolite is excreted through the urine and bile together with trichloroethanol in free or conjugated form. The complete metabolism can be found in the following table:

**Fig. 1.2 Metabolism of chloral hydrate**

In doses used for hypnosis, chloral hydrate produces mild cerebral depression and quiet, deep sleep, usually with little or no hangover effects. Chloral hydrate decreases night time awakening with minimal effects on REM sleep. REM rebound does not occur with drug withdrawal. Tolerance to chloral hydrate may develop over a 5-14 day period of continued use. Chloral hydrate is a carcinogen known to cause cancerous liver tumors in laboratory animals (“Evidence on the Carcinogenicity of Chloral Hydrate,” Reproductive and Cancer Hazard Assessment Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, November 2003).
1.9 FREE RADICALS IN CELLS

A free radical is any atom or molecule that has a single unpaired electron in an outer shell (Erbas and Sekerci, 2011). Once formed, these highly reactive radicals can start a chain reaction. Electrons normally exist in pairs in specific orbitals in atoms or molecules. Free radicals, which contain only a single electron in any orbital, are usually unstable towards losing or picking up an extra electron, so that all electrons in the atom or molecule will be paired (Orchin et al., 2005).

Damage occurs when the free radical encounters another molecule and seeks to find another electron to pair its unpaired electron. The free radical often pulls an electron off a neighboring molecule, causing the affected molecule to become a free radical itself. The new free radical can then pull an electron off the next molecule, and a chemical chain reaction of radical production occurs. The free radicals produced in such reactions often terminate by removing an electron from a molecule which becomes changed or cannot function without it, especially in biology. Such an event causes damage to the molecule, and thus to the cell that contains it. The chain reaction caused by free radicals can lead to cross-linking of atomic structures. Free radicals can be caused by environmental toxins, stress, food additives. They are generated in mitochondria of normal mammalian cells as a byproduct of normal respiration and in other subcellular locations as a function of biochemical reaction using oxygen. Free radicals can attack many different types of molecules within cells, but commonly affect DNA, fats and proteins. Lipids are the most susceptible to free radical damage. The most reactive and damaging free radicals include, hydroxyl radicals, hydrogen peroxide, superoxide, peroxynitrite, nitric oxide, malondialdehyde etc (Hang Cui et al., 2011).

Alcohol and Lipid peroxidation

Lipid peroxidation has been the subject of considerable importance in recent years, with studies on the mechanism of formation of the peroxide radicals and their role in the pathogenesis of several diseases. The peroxidation of polyunsaturated fatty acids, components of biological membranes is considered to be the starting point of many toxic processes (Plaa and Witschi, 1976). The exact mechanism behind the toxic effects of ethanol on hepatocytes is not clearly
understood, but the hepatotoxicity produced by ethanol may be due to lipid peroxidation (Kalish and Di Luzio, 1966) or due to direct physico-chemical effects (Seixas, 1973). Ethanol consumption has been reported to induce alterations in hepatic antioxidant defense capacity, which would result in increased risk of peroxidative damage (Zidenberg et al., 1991). Various reports showed that acute ethanol intoxication decreases the hepatic glutathione content (Vina et al., 1980; Guerri and Grisolia, 1980; Lauterburg et al., 1984; Zentella et al., 1994; Kukielka et al., 1994; Garcia-Ruiz et al., 1995), but there are also reports showing that it is increased (Hetu et al., 1982) and remain unchanged (Sato et al., 1981; Kurose et al., 1996).

Fig 1.3 A diagrammatic representation of free radical activity

**Free radicals and related liver diseases**

An increased production of free radicals in the liver has been implicated in a variety of liver disease. The capability of ethanol to increase reactive oxygen species (ROS) /reactive nitrogen species (RNS) and peroxidation of lipids, DNA, and proteins was demonstrated in a variety of systems, cells and species including humans. ROS/RNS can activate hepatic stellate cells, which are characterized by the
enhanced production of extracellular matrix and accelerated proliferation. ROS play an important role in fibrogenesis throughout increasing platelet-derived growth factor. Free radical initiated lipid peroxidation may play a role in hepatic fibrogenesis, perhaps through an effect of aldehydic peroxidation products on Kupffer cells and lipocytes. Cellular damage in human liver diseases is probably multifactorial and free radicals may play important roles in initiating and/or perpetuating this damage (Britton and Bacon, 1994).

1.10 ANTIOXIDANT DEFENSE SYSTEM

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies Helmut, 1997).

Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating them from free radicals (Halliwell, 2012). They are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidants are helpful in reducing and preventing damage from free radical reactions because of their ability to donate electrons which neutralize the radical without forming another. Ascorbic acid, for example, can lose an electron to a free radical and remain stable itself by
passing its unstable electron around the antioxidant molecule (Bagchi et al., 1997). This has led to the hypothesis that large amounts of antioxidants with their ability to decrease the numbers of free radicals might lessen the radical damage causing chronic diseases (Biesalski, 2002).

The antioxidant enzyme system is very complex, being composed of small molecular weight antioxidant compounds (vitamins E, C, A); primary (superoxide dismutases, catalase, glutathione peroxidase and secondary antioxidant enzymes (glutathione reductase, glucose-6-phosphate dehydrogenase etc); glutathione, glutaredoxin and thioredoxin systems. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997).

**Vitamin E (Tocopherols and tocotrienols)**

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties (Herrera and Barbas, 2001; Packer et al., 2001). Of these, α-tocopherol has the highest bioavailability, with the body preferentially absorbing and metabolising this form (Brigelius-Flohé et al., 1999).

It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. This is in line with findings showing that α-tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death. GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes (Wang et al., 1999).
Ascorbic acid (Vitamin C)

This is a water soluble vitamin which scavenges free radicals present in an aqueous environment. Ascorbic acid is required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline. In other cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide (Padayatty et al., 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka et al., 2002). Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts (Smirnoff and Wheeler, 2000).

Beta-carotene

It is a water soluble vitamin and is the most widely studied of the 600 carotenoids identified. It is thought to be the best quencher of singlet oxygen, an energized but uncharged form of oxygen that is toxic to cells. Beta-carotene is also excellent in scavenging free radicals at low oxygen concentrations.

Melatonin

Melatonin is a powerful antioxidant (Tan Dun-Xian et al., 2007). It is a hormone made by pineal gland, a small gland in brain and is chemically N-acetyl-5-methoxytryptamine. Melatonin easily crosses cell membranes and the blood–brain barrier (Reiter et al., 2009). Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan Dun-Xian et al., 2000).
Fig. 1.4. Enzyme system

Enzymatic pathway for detoxification of reactive oxygen species

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another (Sies, 1997; Davies, 1995).

1.11 SYNERGETIC EFFECT OF ALCOHOL AND DRUGS

Alcohol is the most used recreational substance and one of the most widely abused drugs in the world. Alcohol use is characterized by CNS intoxication symptoms, impaired brain activity, poor motor coordination, and behavioural changes. The impairments in CNS activities are due to alcohol’s effect on synthesis, release, and signaling of neuron transmitters, including glutamate, GABA, and other neuron transmitters (Wallner et al., 2006; Prosser et al., 2008; Wakita et al., 2012). Alcohol use also affects insulin sensitivity that regulates protein, carbohydrate, and fat metabolism (Ting and Lautt, 2006). Chronic abuse of alcohol can result in tolerance and physical dependence. Although significant advances in understanding of alcohol’s effects have been made over the past decades, the pathogenesis of alcohol use and abuse is not fully understood. Understanding the mechanisms that lead to tolerance and dependence may give valuable insight into alcohol addiction and vulnerability and ultimately result in effective therapeutic intervention to facilitate detoxification. (Manzo-Avalos and Saavedra-Molina, 2010).
High levels of alcohol consumption are associated with an increased risk of alcoholism, malnutrition, chronic pancreatitis, alcoholic liver disease, and cancer. In addition, damage to the central nervous system and peripheral nervous system can occur from chronic alcohol abuse (Müller et al., 1985; Testino, 2008). The long-term use of alcohol is capable of damaging nearly every organ and system in the body. Chronic heavy drinking causes the liver to become fatty. This condition makes the liver more vulnerable to dangerous inflammation, such as alcoholic hepatitis, and its associated complications. With continued drinking, persistent inflammation causes fibrous tissue to increase in the liver, which prevents the necessary blood supply from reaching the liver cells. Without the oxygen and other nutrients supplied by this blood, the liver cells eventually die and are replaced with scar tissue, creating a condition known as cirrhosis. In mild cases, the liver can actually make repairs and continue to function. However, advanced cirrhosis causes continued deterioration and liver failure.

The developing adolescent brain is particularly vulnerable to the toxic effects of alcohol (Guerri and Pascual, 2010). In addition, the developing fetal brain is also vulnerable, and fetal alcohol syndrome (FAS) may result if pregnant mothers consume alcohol. Large amount of alcohol over the long term can lead to alcoholic cardiomyopathy. Alcoholic cardiomyopathy presents in a manner clinically identical to idiopathic dilated cardiomyopathy, involving hypertrophy of the musculature of the heart that can lead to congestive heart failure (Awtry and Philippides, 2010). It was estimated in 2006 that 3.6% of all cancer cases worldwide are related to alcohol drinking, resulting in 3.5% of all cancer deaths (Boffetta et al., 2006).

Acetaldehyde, a metabolic product of alcohol, is suspected to promote cancer. Typically the liver eliminates 99% of acetaldehyde produced. However, liver disease and certain genetic enzyme deficiencies result in high acetaldehyde levels. Heavy drinkers who are exposed to high acetaldehyde levels due to a genetic defect in alcohol dehydrogenase have been found to be at greater risk of developing cancers of the upper gastrointestinal tract and liver (Homann et al., 2006).
Alcohol is converted to acetate in the periphery, particularly in the liver (Lundquist et al., 1962; Norberg et al., 2003) and it is released to the blood (Jucker et al., 1998). Administration of ethanol to humans elevates blood acetate (Lundquist et al., 1973; Davin et al., 1994). Consumption of enough alcohol to achieve breath alcohol levels of even 50 mg% is sufficient for plasma acetate levels to approach 1–2 mM, beyond which the plasma acetate concentration does not rise (Lundquist et al., 1962; Nuutinen et al., 1985; Mascord et al., 1992). Acetate travels to other organs, including the brain, for use as an energy substrate (Patel et al., 2010) and in fatty acid and cholesterol biosynthesis (Hellman et al., 1954, Natali et al., 2007). The conversion of ethanol to acetate begins with metabolism to acetaldehyde. In people who consume alcohol at moderate levels or occasionally, the ethanol is metabolized to acetaldehyde in a reversible reaction catalyzed by alcohol dehydrogenase in cytosol, and subsequently acetaldehyde is oxidized to acetate in an irreversible reaction by aldehyde dehydrogenase in mitochondria (Manzo-Avalos and Saavedra-Molina, 2010). In people who are chronic and heavy alcohol consumers, a second pathway becomes prominent, and that is the microsomal ethanol-oxidizing system, which functions in the smooth endoplasmic reticulum of hepatocytes to convert ethanol to acetaldehyde via cytochrome p450. A third route of ethanol metabolism is through catalase, which is located in cell bodies and is capable of oxidizing small amounts of ethanol to acetaldehyde. Acetaldehyde dehydrogenase quickly catalyzes the conversion of acetaldehyde to acetate, and alcohol-dependent individuals show elevated acetate up to 24 hours after the last drink (Pronko et al., 1997). Acetate enters the tricarboxylic acid (TCA) cycle for oxidation via acetyl-CoA synthetase, and the process of oxidation generates adenosine (Kiviluoma et al., 1989; Diamond et al., 1991; Kiselevski et al., 2003;Mailliard and Diamond, 2004), which has sedating properties similar to those of ethanol. One may expect heavy drinkers to have long periods of time with elevated levels of adenosine in their tissues (Carmichael et al., 1991; Carmichael et al., 1993).

In brain, glucose is the major supply of mitochondrial energy oxidation; however, acetate can also be used as an alternative energy source. Heavy alcohol
drinking has been reported to lead to hypoglycemia (Jain et al., 2002). Recent data show that alcohol decreases brain glucose utilization (Volkow et al., 2006; Pawlosky et al., 2010) and increases acetate uptake (Volkow et al., 2013).

**Alcohol-Drug Interaction** (Zakhari, 2006).

Mixing alcohol with other depressant drugs such as benzodiazepines or gamma-hydroxybutyric acid can cause a person’s breathing and heart rate to decrease to dangerous levels and increase the risk of overdose. Drinking alcohol and smoking cannabis together can increase the chances the unpleasant effects, including nausea, vomiting and feelings of panic, anxiety and paranoia. Combining alcohol with stimulant drugs places the body under great stress and can mask some of the effects of alcohol. If a person combines alcohol with energy drinks that contain caffeine (a stimulant) they will still be affected by the alcohol but may not feel as relaxed or sleepy. They may feel more confident, take more risks and increase the chances of experiencing alcohol-related harm such as drinking too much or being injured in a fight or accident.

A drug generally must travel through the bloodstream to its site of action, where it produces some change in an organ or tissue. The drug’s effects then diminish as it is processed (metabolized) by enzymes and eliminated from the body. Alcohol behaves similarly, travelling through the bloodstream, acting upon the brain to cause intoxication, and finally being metabolized and eliminated, principally by the liver. The extent to which an administered dose of a drug reaches its site of action may be termed its availability. Alcohol can influence the effectiveness of a drug by altering its availability.

Typical alcohol-drug interactions include the following:

First, an acute dose of alcohol (a single drink or several drinks over several hours) may inhibit a drug's metabolism by competing with the drug for the same set of metabolizing enzymes. This interaction prolongs and enhances the drug's availability, potentially increasing the patient's risk of experiencing harmful side effects from the drug.
Second, in contrast, chronic (long-term) alcohol ingestion may activate drug-metabolizing enzymes, thus decreasing the drug's availability and diminishing its effects. After these enzymes have been activated, they remain so even in the absence of alcohol, affecting the metabolism of certain drugs for several weeks after cessation of drinking. Thus, a recently abstinent chronic drinker may need higher doses of medications than those required by nondrinkers to achieve therapeutic levels of certain drugs.

Third, enzymes activated by chronic alcohol consumption transform some drugs into toxic chemicals that can damage the liver or other organs.

Fourth, alcohol can magnify the inhibitory effects of sedative and narcotic drugs at their sites of action in the brain. To add to the complexity of these interactions, some drugs affect the metabolism of alcohol, thus altering its potential for intoxication and the adverse effects associated with alcohol consumption.

An interaction between alcohol and a drug is described as any change in the properties or effects of the drug in the presence of alcohol. Drug interactions may be:

- **Additive**: The net effect of the drug taken with alcohol is the sum of their effects.
- **Synergistic**: The effect of the drug when combined with alcohol is greater than the sum of their effects.
- **Antagonistic**: The effect of the drug is diminished in the presence of alcohol.

Since the liver is responsible for metabolizing drugs other than alcohol, potentially dangerous alcohol-drug interactions can occur in both light and heavy drinkers. When ethanol is withdrawn, a withdrawal syndrome appears which is due at least partly to this adaptive state. The withdrawal syndrome in human is effectively treated with diazepam, a drug with agonistic properties at the gamma-aminobutyric acid /benzodiazepine receptor complex. Under these conditions diazepam has anticonvulsant and anxiolytic effect. Recognize that even herbal medicines and supplements can have adverse effect with alcohol (Salloum et al., 1995; Shaw, 1995; Verbanck, 1995).
Table 1.9: Alcohol-drug Interaction

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effects</th>
<th>Interactions with Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana</td>
<td>A 2-4 hour high indicated by bloodshot eyes, slowed motor skills and reaction time, impaired recall, distorted perceptions of time and space.</td>
<td>Exacerbates the sedative effect and increases the level of intoxication of both drugs.</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Mood elevation, euphoria, increased energy, alertness, anxiety, irritability, insomnia, decreased appetite. Alcohol may be used to counteract anxiety and tweaking effects of cocaine.</td>
<td>Potentially very dangerous because alcohol also elevates blood pressure increasing risk for heart attack and stroke.</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>Altered perception of all senses, euphoria, anxiety, depersonalization increased body temperature, heart rate, blood pressure, loss of appetite, sleeplessness.</td>
<td>Unknown, may counteract anxiety.</td>
</tr>
<tr>
<td>Sedatives &amp; Tranquilizers</td>
<td>Effects are similar to alcohol but aggression is less likely lowered, inhibitions, slowed pulse and breathing lowered blood pressure drowsiness.</td>
<td>Severe drowsiness depressed cardiac and pulmonary function which can be fatal.</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Medication may become ineffective and lessen their benefit the side effects from the medication could also worsen. Some antidepressant cause drowsiness and so does alcohol, mixing the two could make you sleepy.</td>
<td>Studies have proved that even social drinking may impair the ability to react quickly and remain alert while driving, even hours after consuming a single alcoholic drink.</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Nausea and abdominal pain are fairly common side effects.</td>
<td>Most antibiotics are less effective when taken with alcohol, may exacerbate nausea.</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Drowsiness, dry mouth.</td>
<td>Severe drowsiness, dry mouth.</td>
</tr>
</tbody>
</table>
Antidiabetic/Hypoglycemic drugs used to treat diabetes and hypoglycemia, such as insulin, combined with alcohol can cause severe and unpredictable reactions. People taking these medications should avoid alcohol at all times. The excess alcohol intake for a long time causes fatty liver and accumulation of fat in the brain, heart and kidneys which agrees with (Ramakrishnan, 1973; Ramakrishnan et al., 1976; Ramakrishnan, 1983).

1.12 METABOLISM OF ALCOHOL

Metabolism is the body's process of converting ingested substances to other compounds. Metabolism results in some substances becoming more, and some less, toxic than those originally ingested. Metabolism involves a number of processes, one of which is referred to as oxidation. Through oxidation, alcohol is detoxified and removed from the blood, preventing the alcohol from accumulating and destroying cells and organs. A minute amount of alcohol escapes metabolism and is excreted unchanged through the breath and urine. Until all the alcohol consumed has been metabolized, it is distributed throughout the body, affecting the brain and other tissues (Wallgren, 1970; Bosron et al., 1993).

![Graph of Blood Alcohol Concentration (BAC) over time](image_url)

**Fig. 1.5.** Blood alcohol concentration (BAC) after the rapid consumption of different amounts of alcohol by eight adult fasting male subjects (Wilkinson et al., 1977).
The major mechanism of alcohol oxidation is a reaction with cytoplasmic enzyme, alcohol dehydrogenase (ADH), with nicotinamide adenine dinucleotide (NAD) as a co-factor. This reaction leads to the formation of acetaldehyde and to the reduction of NAD to NADH. Chronic consumption of alcohol may lead the involvement of two other enzyme systems such as catalase-together with hydrogen peroxidase and the microsomal ethanol oxidizing system (MEOS) as described by Lieber and DeCarli, (1972). Ethanol consumption in man and animals leads to an increase in smooth endoplasmic reticulum (Iseri et al.,1966; Lane and Lieber, 1966; Rubin and Lieber,1967, Rubin and Lieber, 1968; Lieber and Rubin,1968), NADPH oxidase (Lieber and De Carli,1970a) and the enzymes of microsomal ethanol oxidizing system (MEOS) (Lieber and De Carli,1968,1970b).The enzymes of MEOS are localized in the smooth endoplasmic reticulum (SER) and its activity originates from the presence of cytochrome p-450, a mixed function oxidase which utilizes NADPH and molecular oxygen for the oxidation of ethanol (Lieber,1991).

![Fig. 1.6 Ethanol oxidation path way](image)

The acetaldehyde formed by the oxidation of ethanol is converted to acetate by aldehyde dehydrogenase (ALDH), which is also associated with the conversion of NAD to NADH. The acetyl radical combines with Coenzyme A to form acetyl-CoA. The acetyl-CoA then enters the Krebs Cycle, which is the basic powerhouse.
of the human body. Inside the Krebs Cycle this acetyl radical is eventually broken down into carbon dioxide and water.

Cascales *et al.*, (1985) reported that ethanol or acetaldehyde induce alterations in the major hepatic enzymes associated with the metabolism of ethanol such as AST, ALT, NAD-glutamate dehydrogenase etc. A marked increase in the activity of gamma glutamyltranspeptidase after chronic treatment of ethanol was observed, but activities of various other enzymes such as alkaline phosphatase, lactate dehydrogenase etc. were not altered (Antonenkov et al., 1989).

It has been shown that the mean acetaldehyde levels are significantly higher in alcoholics during alcohol metabolism (Korsten et al., 1975). This higher acetaldehyde levels in alcoholism may result from both greater activity of liver microsomal ethanol oxidizing system and mitochondrial damage (Shiohara et al., 1984). This chronic ingestion of alcohol leads to higher levels of plasma free acetaldehyde. This showed that in addition to increased acetaldehyde production, decreased catabolism is also a factor for the higher acetaldehyde levels (Pikkarainen et al., 1981).

Studies showed that mitochondrial aldehyde dehydrogenase may be primarily responsible for the oxidation of acetaldehyde produced during ethanol metabolism (Parrilla et al., 1974). But the role of mitochondrial ALDH in the acetaldehyde metabolism has not yet been clearly established, because administration of high dose of ethanol causes a reduction in the hepatic ALDH activity in the soluble mitochondrial and microsomal fractions of rat liver (Koivula and Lindros, 1975). Studies also showed that acetaldehyde has greater neuronal toxicity than alcohol when applied to cultures of rat sympathetic ganglion cells (Eranko et al., 1977). Stowell et al., (1977) reported that the deprotenization of blood in the presence of alcohol can lead to the production of acetaldehyde. But it is not known, what level of acetaldehyde is sufficient to cause neuronal degeneration and then contribute to the mental deterioration (Acker, 1982).

It was believed that malnutrition is the factor behind the liver diseases of the alcoholics. But further studies showed that alcohol itself has direct toxic effects
upon the liver. The prolonged injury due to ethanol consumption may lead to hepatic lesions such as cirrhosis. The evolution of cirrhosis from fatty liver takes place through a sequence of events, which can be considered as transitional stages between the fatty liver and cirrhosis. These increasing lesions with extensive necrosis and polymorphonuclear inflammation are sometimes considered characteristic of a stage of alcohol hepatitis. Hence the injurious effects of alcohol in the liver are multiple and the classic distinctions between simple fatty liver, alcoholic hepatitis and cirrhosis are very difficult.

**Nonoxidative ethanol metabolism**

In addition to the main oxidative pathways, ethanol is also metabolized, even though to a minor extent, by a nonoxidative pathway to form fatty acid ethyl esters (Lieber, 1997). This reaction is catalyzed by fatty acid ethyl ester synthases and may be of importance in organs lacking ethanol oxidative metabolism, such as the heart. Fatty acid ethyl esters resulting from this nonoxidative ethanol metabolic reaction are involved in alcohol-induced organ injuries (Beckemeier and Bora, 1998).

**1.13 NEUROTRANSMITTER RELEASE AND FUNCTION**

Neurotransmitters are the chemical messengers that are used to communicate between adjacent cells. They are liberated from one nerve cell that impinges other nerve or muscle cells. Acetylcholine is the excitatory neurotransmitter at the neuromuscular junctions. It is synthesized in nerve cells by the enzyme choline acetyl transferase and stored in the synaptic vesicles, which are abundant near the cytoplasmic membrane of the presynaptic axon. After triggering an action potential, the acetyl choline is subsequently degraded into acetate and choline by an enzyme in the synaptic cleft called acetylcholine esterase. In addition to acetylcholine there are a lot of other neurotransmitters such as epinephrine, nor-epinephrine, dopamine, gamma amino butyric acid (GABA) etc. collectively called catecholamines. (Hodgson Ernest, 1932).

It has been reported that the chronic intake of alcohol induces a variety of effects on the metabolism of neurotransmitters and the function of their receptors (Banerjee et al., 1978). Studies on the dependence to alcohol or the functional
tolerance to alcohol have clearly demonstrated that neurotransmitter gated and receptor coupled ion channels are invariably suppressed during alcohol dependence (Kuriyamma and Ohkuma, 1990).

Ethanol induces a variety of alterations in the neurotransmitter function as a result of its disruption of membrane structure and associated electrical properties (Hunt, 1981). Fluidity of the neuronal membrane increases by ethanol administration due to the alteration in the ratio of unsaturated to saturated fats. The resulting change in the membrane structure affects transport process of calcium and other electrolytes, and the active transport of neurotransmitter across the cell surface. This will lead to an impairment of the neurotransmitter receptor function and alter the membrane microenvironment. This will lead to alterations in the cellular function and ultimately behaviour (Leonard, 1986). Ethanol intake produces a multitude of effects on many transmitter systems in the brain (Liljequist et al., 1984; Peoples et al., 1996).

1.14 THE LIVER - ITS STRUCTURE AND FUNCTIONS

The liver is the largest glandular organ and is the central chemical laboratory in the body. It is an organ of paramount importance and plays a pivotal role not only in metabolism and disposition of exogenous toxins and therapeutic agents responsible for metabolic derangement, but also in the biochemical regulation of fats, carbohydrates, amino acids, protein, blood coagulation and immuno-modulation (Ram and Goel, 1999). It is located in the upper right-hand portion of the abdominal cavity, beneath the diaphragm, and on top of the stomach, right kidney, and intestines. Shaped like a cone, the liver is a dark reddish-brown organ that weighs about 3 pounds.

There are two distinct sources that supply blood to the liver, including the following:

- Oxygenated blood flows in from the hepatic artery
- Nutrient-rich blood flows in from the hepatic portal vein

The liver consists of two main lobes, both of which are made up of thousands of lobules. These lobules are connected to small ducts that connect with
larger ducts to ultimately form the hepatic duct. The hepatic duct transports the bile produced by the liver cells to the gall bladder and duodenum.

**Fig. 1.7. Biliary System**

**Functions of the liver:**

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 through 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. Because the liver performs so many vital functions, it is prone to disease. Common liver diseases include hepatitis infection, fatty liver disease, cancer as well as damage from alcohol, the pain reliever acetaminophen, and some cancer drugs

1.15 LIVER TOXICITY

Liver cell injury caused by various toxins such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide etc., chronic alcohol consumption and microbes is well studied.

Hepatotoxins

A number of pharmacological and chemical agents act as hepatotoxins. There are direct and indirect hepatotoxins.

A. Direct hepatotoxins- Agents that damage the membrane of hepatocytes directly result in interference in cell metabolism.

B. Indirect hepatotoxins- Agents that produce hepatic injury as a result of selective interference with metabolic pathways or selective binding to or alteration of a specific component are termed as indirect hepatotoxins.
### Table 1.10 Direct hepatotoxins and their effects

<table>
<thead>
<tr>
<th>Name</th>
<th>Morphological alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon tetrachloride</td>
<td>Decreases glycogen and protein levels and increases the content of lipid</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Decreases glycogen and protein levels and increases the content of lipid</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>Decreases glycogen and protein levels without affecting the lipid level</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>Decreases glycogen and protein levels and increases Lipid profile</td>
</tr>
<tr>
<td>Ethyl Alcohol</td>
<td>Causes hepatocyte degeneration, collagen deposition and necrosis</td>
</tr>
<tr>
<td>Fulvine</td>
<td>Produces edema and congestion and has damaging effect on the parenchyma</td>
</tr>
<tr>
<td>Phalloidin (toxin from Mushroom)</td>
<td>Damages the plasma membrane of the hepatocytes as well as their active filaments</td>
</tr>
</tbody>
</table>

### Table 1.11 Indirect hepatotoxins and their effect

<table>
<thead>
<tr>
<th>Drugs and chemicals</th>
<th>Class of agent</th>
<th>Morphological change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl testosterone</td>
<td>Anabolic steroid</td>
<td>Cholestasis</td>
</tr>
<tr>
<td>Methimazole</td>
<td>Antithyroid</td>
<td></td>
</tr>
<tr>
<td>Erythromycin estolate</td>
<td>Chemotherapeutic</td>
<td></td>
</tr>
<tr>
<td>Chlorpropamide</td>
<td>Oral hypoglycemic</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Tranquilizer</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Chemotherapeutic</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Anticonvulsant</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Antitubercular</td>
<td>Cholestasis and necrosis</td>
</tr>
<tr>
<td>Halothane</td>
<td>Anaesthetic</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Anticonvulsant</td>
<td></td>
</tr>
<tr>
<td>Methyldopa</td>
<td>Antihypertensive</td>
<td></td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Anti-inflammatory</td>
<td>Granulomas</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>Chemotherapeutic</td>
<td></td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Xanthine oxidase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inhibitor</td>
<td></td>
</tr>
<tr>
<td>Yellow phosphorus</td>
<td>Metal</td>
<td>Toxic(necrosis)</td>
</tr>
<tr>
<td>Amanita phalloides</td>
<td>Mushroom</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Analgesic</td>
<td></td>
</tr>
</tbody>
</table>
1.16 MECHANISM OF DETOXIFICATION

There are several enzyme systems involved in biochemical transformations. Two major types of reactions occur in the liver in presence of exogenous substances.

i) In phase I reaction- chemical modification of groups by oxidation, reduction, hydroxylation, sulphonation, dealkylation and demethylation.

ii) In phase II reaction- the conversion of exogenous substances to their glucuronide, sulfate, acetyl, taurine or glycine derivatives to make lipophilic substances to water soluble derivatives and excreted through bile or urine.

iii) Another mechanism by which mixed function oxidase dependent biotransformations produce liver injury is due to formation of activated oxygen species. The cytochrome P-450 system produces hydrogen peroxide from the dismutation of superoxide anions. The oxidative stress imposed by the formation of \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) may offer an alternative to covalent binding as an explanation of biological activity (Ram and Goel, 1999).

1.17 ALCOHOL-RELATED LIVER DISEASES

Liver is a metabolic organ affected by various chemicals and toxins. Chronic alcoholism produces a wide spectrum of liver and other organ diseases depending on the amount and duration of alcohol intake (Soliman et al., 2006). Chronic alcohol intake produces a variety of physiological changes and damages to the liver (Saraswat et al., 1999). Oxidative stress is well recognized to be a key step in the pathogenesis of tissue injury by ethanol generating reactive oxygen species in many tissues. Alcohol consumption for long period decreases the endogenous antioxidants and enhances the lipid peroxidation process (Sadrzadesh et al., 1994) in tissues. Following ethanol intoxication, the balance between prooxidants and antioxidants is disturbed to such extent that results in the oxidative damage of biomolecules such as fat, proteins and finally leading to cell injury (Das and Vasudevan, 2007). Free radicals have been implicated in the causation of liver
cirrhosis and compounds that can scavenge free radicals have great potentials in ameliorating these processes (Wilson, 1998).

Antioxidants and the antioxidant defense system in liver, thus, play an important role to protect the liver against damage by reactive species (Lollinger, 1981).

Administration of ethanol produced a visible severe distortion of the hepatic architecture in the liver. Ethanol consumption induced alteration in the activities of antioxidant enzyme system. Alcohol reduces the levels of antioxidant enzyme activities and promotes the generation of reactive oxygen species (ROS) that leads to liver damage (Dahiruand Oboidoa, 2007; Mallikarjuna et al., 2010; Shanmugam et al., 2011). Ethanol is known to deplete GSH level via the generation of oxidants as well as by inhibiting the mitochondrial glutathione transporter (Wheeler et al., 2003). GSH is a critical cellular antioxidant and is important in limiting the toxicity of ethanol.

The endogenous glutathione peroxidase system and catalase are important antioxidants and cytoprotective machinery in the hepatocyte exposed to ethanol (Kurose et al., 1996). SOD, CAT, and GPX constitute a mutually supportive team of defense against reactive oxygen species (ROS). ROS production and oxidative stress in liver cell play a central role in the development of alcoholic liver disease. Ethanol intoxication disturbs the balance between prooxidants and antioxidants to the extent of inducing oxidative damage to biomolecules leading to cell injury and a decline in the activities of these enzymes (SOD, CAT, GPX, GST and GRD) revealed the oxidative stress elicited by ethanol intoxication (Wu and Cederbaum, 2003).

Alcoholic liver disease is a term that encompasses the hepatic manifestations of alcohol overconsumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis (O'Shea et al., 2010). Although steatosis (fatty liver) will develop in any individual who consumes a large quantity of alcoholic beverages over a long period of time, this process is transient and reversible. Of all chronic heavy drinkers, only 15–20% develops hepatitis or cirrhosis, which can occur concomitantly or in succession. 80% of alcohol passes
through the liver to be detoxified. Chronic consumption of alcohol results in the secretion of pro-inflammatory cytokines, oxidative stress, lipid peroxidation, and acetaldehyde toxicity. These factors cause inflammation, apoptosis and eventually fibrosis of liver cells. Additionally, the liver has tremendous capacity to regenerate and even when 75% of hepatocytes are dead, it continues to function as normal. There are three main types of alcohol-related liver disease: fatty liver disease, alcoholic hepatitis, and alcoholic cirrhosis (Menon et al., 2001).

**Fatty liver disease** (Iseri et al., 1966)

Fatty liver disease is the buildup of extra fat in liver cells. It is the earliest stage of alcohol-related liver disease. There are usually no symptoms. If symptoms do occur, they may include fatigue, weakness, and weight loss. Almost all heavy drinkers have fatty liver disease. However, if they stop drinking, fatty liver disease will usually disappear.

**Alcoholic hepatitis** (Ishii et al., 1997)

Alcoholic hepatitis causes the liver to swell and become damaged. Symptoms may include loss of appetite, nausea, vomiting, abdominal pain, fever and jaundice. Up to 35 percent of heavy drinkers develop alcoholic hepatitis.

Alcoholic hepatitis can be mild or severe. If it is mild, liver damage may be reversed. If it is severe, it will lead to serious complications including liver failure and death.

**Alcoholic cirrhosis** (Lahnborg et al., 1981)

Alcoholic cirrhosis is the scarring of the liver -- hard scar tissue replaces soft healthy tissue. It is the most serious type of alcohol-related liver disease. Symptoms of cirrhosis are similar to those of alcoholic hepatitis. Between 10 and 20 percent of heavy drinkers develop cirrhosis. The damage from cirrhosis cannot be reversed and can cause liver failure. Avoiding alcohol drinking prevents further damage.
1.18 USE OF CULTURED CELLS TO STUDY ALCOHOL METABOLISM

The association between alcohol abuse and liver disease has been recognized for centuries. The most abundant cell type in the liver, the hepatocytes, metabolizes the vast majority of ingested alcohol; thus, ethanol metabolism is thought to be primarily responsible for ethanol-induced liver damage. Hepatocytes metabolize ethanol through two major metabolic pathways. The primary pathway is mediated by alcohol dehydrogenase, an enzyme located in the cytoplasm of hepatocytes. The cytoplasm is the semi-fluid part of the cell located between the
cell membrane and the nucleus. The other pathway is mediated by cytochrome p450 2E1, an enzyme bound to the network of membranes within the cell known as the endoplasmic reticulum (Clemens, 2006).

Ethanol metabolism results in a number of biochemical changes, including the production of the toxic byproduct acetaldehyde, the production of cell-damaging reactive oxygen and nitrogen species, deficiency of oxygen in the tissues, and an increased ratio of the reduced form of the coenzyme nicotinamide adenine dinucleotide (NADH) to the oxidized form of nicotinamide adenine dinucleotide (NAD\(^+\)). Cells produce NAD from niacin and use it to transport electrons in redox reactions, in which atoms either gain electrons or lose electrons. During this process NAD gains a pair of electrons and a proton and thus is reduced to NADH, releasing one proton. All of these biochemical changes have been proposed to contribute to hepatocyte injury and liver disease, although no single change can account for all of the effects of ethanol metabolism on the liver. In fact, in many cases two or more biochemical changes may act in concert to produce specific effects (Chen and Cederbaum, 1998).

The molecular mechanisms of ethanol-induced liver damage have been difficult to determine because these biochemical changes occur simultaneously. Therefore, using animal models, it has been extremely difficult to establish a cause-and-effect relationship between specific biochemical changes and specific pathologic changes.

The effects of ethanol metabolism on the liver can broadly be placed into three categories: (1) effects on hepatocyte functions, (2) effects on hepatocyte viability, and (3) effects on hepatocyte replication. Cell culture models have been used to investigate these effects in detail, providing insight into the molecular mechanisms of ethanol-induced liver damage.

**Effects of Ethanol Metabolism on Hepatocyte Functions**

The liver is responsible for the removal of many toxic substances from the blood, as well as the synthesis and secretion of many compounds. The most abundant cells in the liver, the hepatocytes, primarily carry out these functions. It
has long been known that one of the biological consequences of chronic ethanol consumption is enlargement of the liver (i.e., hepatomegaly). The mechanism of this increase in liver volume is likely complicated and multifactoral, but two factors that may contribute to this change are impaired movement of proteins around a cell (i.e., protein trafficking) and impaired protein degradation.

In many cells that are organized into tissues, the cell membrane is not uniform (i.e., exhibits membrane polarity); this is because different domains of the cell membrane have different functions. Hepatocytes are no exception. Their basal lateral domain of the cell membrane is exposed to the liver sinusoid, or the blood supply, as well as the narrow intercellular space between adjacent hepatocytes, and their apical domain of the cell membrane is exposed to the tube or space between liver cells that collects bile from the cell (i.e., the bile canaliculus). Many cells, including hepatocytes, lose their polarity in culture. To circumvent this problem, Shanks and colleagues (1994) created a polar cell line with hepatic characteristics. The resulting WIF-B cell line expressed both alcohol dehydrogenase and cytochrome p450 2E1, maintained polarity, and formed functional bile canaliculi (Shanks et al., 1994). These cells have been used to investigate the effects of ethanol metabolism on the functions of protein structures that give the cell its shape and facilitate the movement of proteins and organelles throughout the cell (i.e., microtubules). Microtubules are required for a number of critical cellular functions, including organized intracellular protein trafficking. Culturing WIF-B cells in the presence of ethanol for 3 days reduced microtubule polymerization. Morphologically, the microtubules in cells that were cultured in the presence of ethanol appeared gnarled and shorter, characteristics commonly associated with stable microtubules. Analysis revealed that the major component of microtubules, a-tubulin, isolated from ethanol-treated cells was more highly altered by the introduction of an acetyl group (i.e., acetylated) compared with cells cultured in the absence of ethanol. Using specific biochemical inhibitors of alcohol dehydrogenase and aldehyde dehydrogenase, it was shown that ethanol metabolism was required for these changes in microtubules and that acetaldehyde most likely mediated these changes. These alterations in microtubule dynamics could alter
their ability to facilitate protein movement and could be one of the mechanisms responsible for the impairment in protein trafficking observed in alcoholic liver disease (Kannarkat et al., 2006).

**Figure 1.10** Schematic representation of the domains of the hepatocyte membrane. The cell membrane of a liver cell (i.e., hepatocyte), like other cells, is not uniform (i.e., exhibits membrane polarity). Different domains of the cell membrane have different functions. The basal lateral domain of the membrane is exposed to the liver sinusoid, or the blood supply, as well as the narrow intercellular space between adjacent hepatocytes, and the apical domain of the membrane is exposed to the tube or space between liver cells that collects bile from the cell (i.e., the bile canaliculus).

**Toxic Effects of Ethanol Metabolism on Hepatocyte Viability**

Hepatic cells genetically engineered to metabolize ethanol have been extremely valuable tools in investigating the mechanisms by which ethanol damages hepatic cells. Using these cells, one of the first things that became evident was that ethanol itself is not normally toxic to hepatic cells, even at concentrations much higher than those normally detected in the blood of human beings consuming alcohol. Because ethanol itself does not normally cause hepatic cellular toxicity, studies have focused on the toxic effects of ethanol metabolism. The development of the intragastric model of ethanol administration, in which ethanol is directly pumped into the stomach of experimental animals, revealed that enhanced cytochrome p450 2E1 activity was associated with more severe liver disease, implicating cytochrome p450 2E1 activity as a mediator of cell damage. (Dai et al., 1993).
Free radicals also may have a role in the scarring of the liver (i.e., fibrotic response). Using cocultures of E47 cells and the star-shaped liver cells involved in the development of fibrosis (hepatic stellate cells), Nieto and colleagues (2002) showed that the production of reactive oxygen species increased a number of indices associated with activation of stellate cells, including the increased production of collagen type I, a major component of the fibrotic scarring associated with alcoholic liver disease.

One of the first pathologic changes to the liver, in response to ethanol consumption, is accumulation of fatty acids and triglycerides, a condition known as fatty liver. Fatty liver once was thought to be a benign change to the liver but is now recognized as a precursor to a liver condition known as nonalcoholic steatohepatitis (NASH). Because of this, the alcohol-associated accumulation of fat in the liver has received considerable attention. Cultured cells have been used very effectively to help unravel the mechanisms by which fat accumulates in the liver as a result of ethanol metabolism and successfully used to investigate a wide variety of ethanol-induced effects on the liver (Galli et al., 1999).

Cultured hepatoma cells also have been used to investigate the liver-damaging effects of nonoxidative metabolism of ethanol. Nonoxidative ethanol metabolism is mediated by a group of enzymes known as fatty acid ethyl ester synthases. The involvement of nonoxidative ethanol metabolism in the presence and absence of oxidative ethanol metabolism using parental and recombinant Hep G2 cells were investigated and found that fatty acid ethyl ester synthesis was increased in cells that did not express alcohol dehydrogenase and that the increased accumulation of fatty acid ethyl esters was associated with increased apoptotic cell death (Wu et al., 2006).

1.19 APOPTOSIS

Apoptosis or programmed cell death is a normal component of the development and health of multicellular organisms. The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally
and temporally defined self-destruction. Cells of an adult organism constantly undergo physiological cell death which must be balanced with proliferation in order to maintain homeostasis in terms of constant cell numbers (Lockshin and Williams, 1964).

Apoptotic processes are of widespread biological significance, being involved in e.g. development, differentiation, proliferation/homoeostasis, regulation and function of the immune system and in the removal of defect and therefore harmful cells. Thus, dysfunction or dysregulation of the apoptotic program is implicated in a variety of pathological conditions. Defects in apoptosis can result in cancer, autoimmune diseases and spreading of viral infections, while neurodegenerative disorders, AIDS and ischaemic diseases are caused or enhanced by excessive apoptosis (Fadeel et al., 1999a).

The term apoptosis had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr et al., 1972. The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of cell populations in tissues in physiological and pathological conditions.

**Morphological features of apoptosis**

Apoptotic cells can be recognized by stereotypical morphological changes: the cell shrinks, shows deformation and loses contact to its neighbouring cells. Its chromatin condenses and marginates at the nuclear membrane, the plasma membrane is blebbing or budding, and finally the cell is fragmented into compact membrane-enclosed structures, called 'apoptotic bodies' which contain cytosol, the condensed chromatin, and organelles. The apoptotic bodies are engulfed by macrophages and thus are removed from the tissue without causing an inflammatory response. Those morphological changes are a consequence of characteristic molecular and biochemical events occurring in an apoptotic cell, most notably the activation of proteolytic enzymes which eventually mediate the cleavage of DNA into oligonucleosomal fragments as well as the cleavage of a
multitude of specific protein substrates which usually determine the integrity and shape of the cytoplasm or organelles (Saraste and Pulkki, 2000). Apoptosis is in contrast to the necrotic mode of cell-death in which case the cells suffer a major insult, resulting in a loss of membrane integrity, swelling and disruption of the cells. During necrosis, the cellular contents are released uncontrolled into the cell’s environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue (Leist and Jaattela, 2001).

Fig 1.11 Hallmarks of the apoptotic and necrotic cell death process. Apoptosis includes cellular shrinking, chromatin condensation and margination at the nuclear periphery with the eventual formation of membrane-bound apoptotic bodies that contain organelles, cytosol and nuclear fragments and are phagocytosed without triggering inflammatory processes. The necrotic cell swells, becomes leaky and finally is disrupted and releases its contents into the surrounding tissue resulting in inflammation (Modified from Van and Van, 2002).

Initiators and executioners of apoptosis

Caspases are proteins which are of central importance in the apoptotic signaling network. These are activated in most cases of apoptotic cell death. The two main pathways of apoptosis leading to caspase activation are extrinsic and intrinsic apoptosis pathways.
1.20 LIVER AND XENOBIOTIC METABOLISM

The liver which occupies the pivotal position in body plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign dysfunction. Despite the tremendous strides in modern medicine, there is hardly any drug that stimulates liver function, offers protection to the liver from damage or helps regeneration of hepatic cells. The liver protects the body from potentially injurious substances (endotoxins) absorbed from the intestinal tract, as well as the toxic byproducts of metabolism. The most important in the detoxification process is that of the microsomal drug metabolizing system of the liver (Ruch et al., 1989). A large number of xenobiotics are reported to be potentially hepatotoxic. Some examples are acetaminophen, tetracycline, antineoplastic agents, ethanol and carbon tetrachloride. Hepatotoxins may react with the basic cellular constituents – proteins, lipids, RNA and DNA and induce almost all type of lesions of the liver (Guillouzo, 1998).

The effect of a mega dose of ascorbic acid (200 mg/100 g body wt.) on alcohol-induced toxicity in rats was evaluated. In rats administered, alcohol and ascorbic acid, malondialdehyde (MDA), hydroperoxide and conjugated dienes decreased in comparison with that given alcohol alone. The reduced activities of scavenging enzymes, e.g. superoxide dismutase (SOD) and catalase, in ethanol-administered rats were also enhanced by the co-administration of ascorbic acid and ethanol. Co-administration of ethanol and ascorbic acid reduced phospholipids and MDA levels of the erythrocyte membrane in comparison with that of the ethanol fed rats. The reduction in the activities of glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), gamaglutamyltranspeptidase (GGT) and the decrease in triglycerides levels also clearly showed the protective action of ascorbic acid in reducing ethanol induced toxicity (Suresh et al., 2000). The combination of ethanol and diazepam elicits additive or supra-additive pharmacological effects in animals and humans. (Linnoila and Mattila, 1973 a, b; Linnoila et al., 1974; Linnoila and Hakkinen, 1974; Morland et al., 1974).
About the Thesis

This thesis incorporates a comparative study of natural and adulterated toddy. The toxicological effects of common adulterants of toddy such as diazepam, phenobarbitone and chloral hydrate can be ascertained in experimental animals. The study pertaining to the damaging effects of these adulterants on cultured normal liver cell line also forms a portion of the thesis.

The toxicological effects of these adulterants can be ascertained by following the parameters such as liver marker enzymes, lipid peroxidation products and also the enzymic and nonenzymic antioxidant status. Accordingly, in this study the following parameters were analyzed in different groups of experimental rats such as pairfed control, natural toddy treated group, shop toddy treated groups, adulterants alone and co-administered groups. The parameters include changes in the activities of enzymes like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and changes in the level of protein, lactate dehydrogenase (LDH), acetyl cholinesterase in the serum of various groups of rats.

The free radicals generated can induce peroxidative changes. The following aspects were also studied.

Changes in the level of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), glutathione (GSH), activities of catalase (CAT), glutathione-S-transferase (GST) in the liver and kidney of various groups of treated and pairfed control male albino rats.

In addition to the above biochemical parameters, Scanning electron microscopic and Transmission electron microscopic analysis of the brain samples of various treated groups and control groups and histopathological analysis of liver were also carried out.

Apoptosis was studied following the administration of natural toddy, shop toddy and various adulterants alone and in combination in normal Chang liver cells.

The results of these investigations are discussed in this thesis.