CHAPTER I

INTRODUCTION

The concerns regarding alcohol* consumption during pregnancy have their origin in the Bible. "Behold, thou shalt conceive, and bear a son: and now drink no wine or strong drink..." (Judges 13:7). The recipient of this admonition was the mother of Samson. In the medical literature of 18th and 19th century, a large number of papers, reviewed by Warner and Rosett (1975) (1), indicate that the knowledge that prenatal and maternal drinking could damage the fetus, was wide spread, within the medical profession of that time. The most outstanding historical case of public and political awareness on alcohol's ramification upon offspring occurred during Britain's gin epidemic of the 18th century. The British parliament passed a legislation that encouraged the growing and distillation of grains. The government expected that the trade of distilling would substantially increase the revenue of both the land owners and the British treasury due to the export of

* The term Alcohol and ethanol will be used interchangeably
gin. Due to the abundant supply of gin, it became extremely cheap and the poor of Britain became the principal consumers. Poverty and criminality increased with this unlimited sale of gin. (2) In addition to this, there was a decline in London's population. Only one child in four who was born in London between 1730 and 1749 reached the age of five (3). This gin epidemic seems to have offered the doctors great opportunities to publish case reports in this matter. Magnus Huss, a professor of internal medicine in Stockholm, published a famous book on alcoholismus chronicus in 1849 (4), which contains several distinct case reports on congenital defects in offsprings of alcoholic mothers. In 1880 Gerhard Westfelt (5) published a large review article in Swedish on the influence of alcohol on the progenies. William Sullivan, a physician to a Liverpool prison, published a study in 1899 that may have been the first study on maternal alcoholism (6). He examined 600 offspring of 120 jailed drunkards. In contrast to non-alcoholic female relatives, the alcoholic women had still birth and infant mortality rate that was 56% higher than the comparison group. Paternal drinking habit did not appear to affect the mortality rates. If women were forced to abstain from alcohol during pregnancy - as might happen if she were imprisoned - the cycle of adverse outcome was reported to be broken.
Relatively little work was reported in the first half of this century. The characteristics of fetal malformations of children born to alcoholic mothers were first described in detail in 1969 by Lemoine et al (7). Jones and co-workers independently published an almost identical description of the symptomology of this syndrome (8). In 1973 Jones and Smith (9) described the anomalies in children as Fetal Alcohol syndrome (FAS).

Alcohol as a teratogen has been identified as causing

- Fetal Alcohol Syndrome, where deleterious effects are pronounced (9-11) and
- Fetal Alcohol Effects (FAE), where deleterious effects are less distinct (12-14)

The specific symptoms of FAS can be grouped together as indicated in table 1.

<table>
<thead>
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<th>Table 1.</th>
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<td>1. Prenatal and postnatal growth deficiency.</td>
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<td>2. Characteristic facial appearance.</td>
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<td>3. Reduced CNS performance including mental deficiency.</td>
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<td>4. Increased frequency of major anomalies.</td>
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The children suffer from a prenatal onset growth deficiency and they continue to grow poorly in postnatal life for
years. They have a very typical facial appearance, reduced central nervous system performance including mental retardation and an increased frequency of other major anomalies.

Table 2.

<table>
<thead>
<tr>
<th>Performance</th>
<th>Prenatal growth deficiency</th>
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<tr>
<td></td>
<td>Postnatal growth deficiency</td>
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<td></td>
<td>Developmental delay</td>
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<tr>
<td>Craniofacies</td>
<td>Microcephaly</td>
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<td></td>
<td>Short palpebral fissures</td>
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<td>Epicanthal folds</td>
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<td>Maxillary hypoplasia</td>
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<td>Cleft palate</td>
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<td>Micrognathia</td>
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<td>Limbs</td>
<td>Joint anomalies</td>
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<td></td>
<td>Altered palmellar crease pattern</td>
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<td>Others</td>
<td>Cardiac anomalies</td>
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<td></td>
<td>Anomalous external genitalia</td>
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<td></td>
<td>Capillary hemangioma</td>
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<td>Fine-motor dysfunction</td>
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The characteristic features are indicated in more detail in Table 2. Most characteristic feature of FAS is the typical facial appearance with a broad mid-face, a broad nasal bridge with epicanthal folds, an up-turned nose, a small mandible and small eyes (7,8,15-20). The three characteristics initially used in defining FAS are prenatal growth deficiency, microcephaly and other anomalies. (21) Currently, the three features required for diagnosis of full FAS are growth retardation, cutaneous facial abnormalities and CNS dysfunction (22).

Conversely, in FAE there is a continued ethanol effects, which was demonstrated in a study using pigtailed macaques (23). Children display one or more characteristics of prenatal ethanol exposure, but without severe malformations. Generally, FAE is observed in children exposed to less frequent or smaller amounts of ethanol during prenatal development. There are no established criteria diagnosing FAE as exist for FAS (24). Another expression for alcohol related birth defects (ARBDs) have also been coined to indicate reduced birth weight, developmental delay or cognitive dysfunction. Previous studies on ARBDs has shown that several neuroendocrine systems are altered by prenatal exposure to alcohol (25-31).
The incidence of FAS has been estimated to be between one or two per 1000 live births in United States (32). The incidence of FAS is now estimated to be 0.97 cases per 1000 live births in the general obstetric population and 4.3% among heavy drinkers. The general incidence is more than 20 times higher in the United States (1.95/1000) compared to Europe and other countries (0.08/1000). Within United States the incidence is 10 times higher in low socio-economic status (33). FAS is accepted as a leading cause of mental retardation in USA (12). The incidence of FAE is more difficult to determine due to the lack of criteria for diagnosis (24). For every 1000 live births, it has been suggested that between three and five children will exhibit FAE. However the incidence of FAS or FAE could vary widely depending on the specific population studied (32). Jacob et al (34) reported that in Alaska there is high alcohol abuse during pregnancy.

According to Rosett and Weiner (35) ethanol abuse imposes a risk upon the fetus throughout gestation as the development and growth of various systems proceed. As the FAS includes so many diverse aspects of fetal development, it is not unexpected that the full syndrome is only seen in offspring of women who have used alcohol throught their pregnancy. According to Majewski (36) among children born to the same alcohol-abusing
mother the younger siblings were more affected than the older siblings. This reports the increasing risk among alcoholic women with successive pregnancies. Recently Van Duijin reported that intrauterine alcohol exposure increases the risk for childhood acute non-lymphatic leukemia (37).

The minimum level of ethanol consumption which will result FAS or FAE remains to be firmly established. Children with full FAS are born to mothers defined as chronic alcoholics (38). It has been suggested that pregnant women who engage in binge drinking, particularly during the first trimester, are inflicting fetal developmental damage. Consumption during this period has been linked with both fetal death and miscarriage (39). Particularly from the second to eighth week the embryo is most susceptible to teratogens (40). The most critical period for human CNS development is the first 85 days of gestation (41). Further studies proved that the critical period of alcohol teratogenicity in humans is shortly after conception (42). Ethanol exposure is most damaging during gastrulation than in any other developmental period (42-46). Moderate or occasional heavy binge drinking during this period may have serious repercussions for the fetus. Later consumption can also affect the previously formed organs. Near term, ethanol consumption
will likely have greater effect on maternal nutritional state negatively affecting the size of the fetus (47).

The smallest quantity of ethanol ingestion reported to be associated with the FAS is about 75 ml (2.5 oz) daily (48). Although it is not clear whether there is safe lower limit, there is no evidence for any adverse effects associated with modest consumption of alcohol (eg. a single daily glass of wine; 15 ml (0.5 oz of alcohol) (49). The difficulty in establishing the minimal safe level of ethanol intake is due to additional interactive factors such as displacement of other nutrient sources from the diet by alcohol.

*Mechanism of in utero action of ethanol*

Although maternal and fetal circulations are mutually independent, ethanol crosses the placenta readily (50-52). Maternal and fetal blood levels are approximately equal after maternal ingestion. Fetal hepatic enzymes, particularly alcohol dehydrogenase, are immature and present at lower levels. So ethanol is passed back through the placenta for the mother's body to metabolize (24,53). Thus the levels of ethanol stay equal until all ethanol is eliminated. Hayashi (54) reported that administration of ethanol in pregnant rats showed a ten fold increase of acetaldehyde
in mother's blood than in the fetal blood, but the fetal blood and amniotic fluid showed a variable concentration of acetaldehyde after administration.

The mechanisms by which ethanol acts as a teratogen are not clearly understood, but different mechanisms have been proposed:

1. Impaired placental transport.
2. Abnormal muscle organogenesis.
3. Fetal hypoxia.
5. Altered hormone metabolism.

The first mechanism suggests that ethanol impedes placental transport of nutrients. There are reports suggesting that the essential amino acid transport across the placenta is reduced (55-57). A second mechanism by which placental transport may be impaired is by the effect of ethanol on placental enzymes, specifically Na\(^+\)-K\(^+\) ATPase. The decrease in the activity of this enzyme would decrease the active transport of amino acids and sugars, and in turn, inhibit fetal growth (58-59). A second mechanism by which ethanol may cause teratogenesis is through abnormal development of muscle filaments (60). FAS and FAE may
in part result from structural abnormality in cytoskeletal proteins if cell movement is inhibited during fetal development. Fetal hypoxia has also been suggested as a cause of aberrations in structure, physiology, and biology of infants with FAS and FAE (12). Alcohol induced fetal hypoxia has been established. When ethanol is present in the blood stream, the liver can consume upto 100% more oxygen than is normal due to need for oxygen in the process of ethanol metabolism (61). If blood flow does not increase to other tissues, oxygen deprivation will ensue as a result of the elevated oxygen demand. Ethanol ingestion is followed by the release of catecholamines from adrenals (62,63). This will lead to vasoconstriction (24), which exacerbates the oxygen deficiency. Therefore it is possible that hypoxia may cause some anomalies of FAS and FAE. Alcohol causes enhanced release of prostaglandins from various tissues (63-67) as well as interfering with principal catabolic enzymes (68,69). Both actions of ethanol increase the levels of prostaglandins in the fetal circulation. The elevation of prostaglandin levels in turn stimulates cAMP production. When present at high levels, cAMP has been shown to slow the rate of cell division (70). Increased levels of brain cAMP could affect the development of CNS. The exact interaction between ethanol, prostaglandin levels and fetal abnormalities is yet not defined. It
has been suggested that ethanol may elicit deleterious effects in utero by altering maternal and fetal hormone levels during pregnancy (12). Halmesmaki et al (71) found that women giving birth to FAS infants had low levels of estradiol and estriol throughout pregnancy. Low levels of progesterone from the 16th to 24th week and high prolactin concentration during week 16 to 24 was also noted. It is not certain if these deviations from normal concentrations during pregnancy contribute to FAS. Tritt et al (72) is of the opinion that the effect of ethanol on fetal growth is mediated through the effects of ethanol induced changes in the function of the maternal adrenal cortex.

Thus by disturbing various metabolic processes, alcohol acts as a severe teratogen during pregnancy.

Beverages

Of all the additive substances used or misused by man, alcohol is the one most commonly found in nature. The word "alcohol" is derived from the Arabic term 'al-khol', meaning "finely divided spirit", since vapours of fermented products were removed in an invisible form. Alcoholic beverages have been used since the dawn of history, beginning with fermented beverages of relatively
low alcohol content. There is evidence that alcoholic beverage has its beginning very early in civilisation. Twenty three centuries before the dynasty of King Tutunkhamen there was brewery in Egypt. The writings of ancient Chinese and Indian cultures of 2000 B.C. include discussions of alcohol. (38). There is mention in the ancient books of the old Hindu medicine of chronic alcoholism and even delerium tremens. The Aryan invaders of India who appeared to have been well-versed in the art of brewing used a beverage called "somaras". In ancient Greece, where social gatherings were termed symposia meaning "drinking together". About 900 B.C. Lycurgus, King of Sparta tried to control alcoholism by decreeing that wineyards be destroyed and legs of drunkards be cut off (73).

The direct and indirect cost of human life due to alcohol abuse is greater than any other known disease. The total alcohol consumption continues to increase, as does the percentage of the population using it (74). In USA it is an immense problem and out of 80 million people who use alcohol, about 0.7 million are chronic alcoholics and constitute major social and medical problem. New addicts to alcohol are said to be added at the rate of 2000,00 a year. It has been estimated that one-fifth to one-third of all alcoholics in that country are women (73). The major intoxicating agent in alcoholic beverages is ethyl alcohol. Alcohol is a food, a
drug or a poison. This is decided by the nature of the beverage, dosage, frequency of intake and condition of the body (75).

Basically there are 3 classes of alcoholic beverages.

1. Wines and beers that result from an alcoholic fermentation of a sugar containing fruit juice or plant extract. Its alcohol content is 2-3% (V/V).

2. Fortified wines such as sherry, port and vermouths, which are manufactured by adding distilled spirit, usually brandy to a fermenting fruit juice. Alcohol content is around 20% (V/V).

3. Distilled spirits, made as a result of a batch or continuous distillation of fermented fruit juice or plant extract. Potable distilled spirits are usually retailed with an alcohol content of around 40-50% (V/V) (76).

Both non-distilled and distilled liquors are consumed in India. For the purpose of excise law in India, alcoholic drinks have been divided into two categories, country liquors and foreign liquors. The country liquors are plain spirits made from approved bases; they also include fermented liquors prepared
according to indigenous processes. The foreign liquors include all imported liquors and also those manufactured in imitation of the imported liquors. The raw material for making country liquor are mollases, sugar, palmara jaggery, cereals, and Toddy, and sometimes juices tapped from date \((Phoenix dactylifera Linn.)\), palmyra \((Borassus blabellifer Linn.)\), coconut \((Cocos nucifera Linn.)\) and nipa \((Nipa fruticans Wurmb.)\). The foreign spirits produced in India are whisky, brandy, rum and gin. Imitation gin is produced from molasses or gur spirit by flavouring with gin essence (73).

The relatively richer section of the community particularly in urban areas, drink foreign liquors either imported or Indian manufactured. The less well-to-do section of the community generally drink country liquors, both distilled and fermented (73).

**Congeners**

Ethyl alcohol is the major intoxicating agent in all the beverages. Non alcoholic portions of beverages contain pharmacologically active compounds, which may be either hepato toxic or hepato protective. These compounds are known as congeners. Congeners in alcoholic beverages include mainly primary alcohols other than ethanol, aldehydes and esters and
unfermented sugars. To a large extent these substances are responsible for the special aroma and flavour of different wines, beers, and liquors. Frankel et al reported that low concentrations of resveratrol in wines reduce human LDL oxidation (77) and platelet aggregation (78). Vinson and Hontz (79) reported that phenols present in wines are responsible for its antioxidant properties. Red wines had a significantly higher antioxidant index than white wines and thus are a better source of antioxidants. Zariwala et al reported that country liquors have higher carcinogenic potential (80).

Much of the research on the actual role of congeners has been done in studies comparing the relative effects of vodka and bourbon which have respectively, the lowest and highest congener contents of contemporary liquors. According to Carrol (81), the total congener content of vodka is 3.30 mg/100 ml while that of bourbon is 285.56 mg/100 ml. Blum et al (82) found bourbon to produce significantly greater "falling" and "sleeping" effects in mice than vodka. Di Luzio (83), in a study on tissue glyceride concentration, found that 23% of the mice on bourbon died while none of those on comparable doses of ethanol or vodka died. Henry (84) in studying effects of alcoholic beverages containing large and small amounts of congeners on blood pressure and Electro Encephalo Graph had suggested that congeners in alcoholic
beverages have significant pharmacological effects. Ryback and Dowd (85) found bourbon to cause greater and more persistent alcoholic nystagmus than comparable doses of ethanol.

Raynes and Rayback (86) compared the effects of ethanol, bourbon and a congener solution on the aggressive response of the male Siamese fighting fish Betta splendens and, found a significant increase with ethanol and a significant decrease with bourbon and congener solution. The congener solution was having a greater depressent effect than the bourbon. They concluded that the intermediate effect of bourbon was due to the interaction of the depressent effect of congeners with the "disinhibiting" effect of ethanol. In humans, Katkin et al (87) compared the effects of vodka and bourbon on psychomotor tasks and decision making and found little difference on the immediate performance (1 hr after ingestion) but significantly greater impairment of function with bourbon after 5 hr. They concluded that congeners increase ethanol impairment of psychomotor performance and increase risk taking. Gavaler (88) reported the effects of phytoestrogen congeners of alcoholic beverages on estrogenisation of postmenopausal women.

Higher alcohols comprising about two thirds of all the congeners, contribute a good proportion of total congener
effect. Haggard et al (89) found that ethanol might be more slowly metabolised in the presence of congeners. Green berg (90) found that \textit{in vivo} metabolism of isoamyl alcohol to valeraldehyde and disappearance of valeraldehyde from plasma were retarded in the presence of ethanol. But Chopra (91) and Fazakas-Todea et al (92) is of opinion that beverage congeners had no potentiating effects. Abel reported that intra uterine growth retardation after alcohol exposure is not by congeners, but it is caused by ethanol (93).

At this point, the evidence suggests that ethanol and higher alcohols interfere with each others metabolism, thus potentiating the existing additive effect.

\textit{Objectives of the present investigation}

Review of the literature reveals that studies on \textit{in utero} exposure of alcohol/alcoholic beverages were mainly concentrated on function of CNS and behavioural problems. Systematic studies on the development of fetus and its various metabolic processes are scarce. Only few studies have been done using alcoholic beverages. In actual practice people consume alcoholic beverages and not pure ethyl alcohol. In South India the two popular alcoholic beverages are Arrack and Toddy. These beverages are consumed by people belonging to low socio-economic
status. Detailed studies after *in utero* exposure of these beverages is lacking. So in order to understand the role of congeners in these alcoholic beverages we have studied the effect of Toddy and Arrack and their equivalent quantity of alcohol on maternal reproductive performance, on fetus (teratogenic effects), lipid metabolism, carbohydrate metabolism, liver function and hepatic changes.