REVIEW OF LITERATURE
Drug and ethanol seeking behavior has become a great global problem affecting millions of inhabitants with a cost to society in the billions. The etiology of ethanol dependence is a complex interaction of psychosocial and biologic factors (Konishi et al., 2004). The central nervous system (CNS) plays an important role in the peripheral regulation. Neurotransmitters mediate rapid intracellular communications not only within the central nervous system but also in the peripheral tissues. They exert their function through receptors present in both neuronal and non-neuronal cell surface that trigger second messenger signalling pathways (Julius et al., 1989). Central nervous system believed to be mediated by non-specific physicochemical effects on the membrane or by actions through specific receptors (Deitrich et al., 1989; Eckardt et al., 1998). Determining the specific neurotransmitters and receptor subtypes that may be involved in the development and effects of ethanol abuse is the first step in developing medications to treat ethanol addiction (Hunt, 1993; Deitrich & Erwin, 1996).

Central Nervous System and Ethanol

The etiology of ethanol dependence is a complex interaction of psychosocial and biologic factors (Konishi et al., 2004). The effects of ethanol on the brain result mainly from its action on the postsynaptic receptor sites for various neurotransmitters. Heavy ethanol consumption has both immediate and long-term detrimental effects on the brain and neuropsychological functioning (Delin & Lee, 1992; Evert & Oscar-Berman, 1995). Ethanol interferes with communication between nerve cells and all other cells, suppressing the activities
of excitatory nerve pathways and increasing the activities of inhibitory nerve pathways. Central nervous system has a crucial role in ethanol addiction, several actions believed to be mediated by non-specific physicochemical effects on the membrane or by actions through specific receptors (Deitrich et al., 1989; Eckardt et al., 1998). Chronic and excessive consumption of ethanol in humans and animals has been shown to cause cellular damages in many body organs, including neurons and glial cells in the central nervous system (Miller, 1992; Hunt, 1993; Luo & Miller, 1998). Biogenic amines have been implicated in the regulation of aggression (Kravitz, 2000) and memory (Hasselmo, 1995). Brain serotonin (5-HT) modulates the neural and behavioural effects of ethanol in a manner that remains poorly understood (Daws et al., 2006). Ethanol-induced changes in thyroid function may contribute to the development of mood disorders (Liappas et al., 2006). Ethanol ingestion for short as well as long time has been shown to induce significant changes in neurotransmitter systems (Imperato & Di Chiara, 1986; Samson & Harris, 1992), among these DA and 5-HT have received special attention because of their putative role in the motivational effects of ethanol (Cloninger, 1987; Sellers et al., 1992; Wallis et al., 1993). Changes in central DA neurotransmission are implicated in processes as diverse as muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral DA mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release. In the adolescent brain, drinking cessation can partially ameliorate the ethanol-induced morphological changes on neurons and astrocytes but cannot fully return it to the basal state (Evrard et al., 2006). DA itself has a regulatory effect on the synthesis of post-synaptic receptors. Schizophrenia causes an increased DA D₂ receptor synthesis due to dopaminergic blockade by neuroleptics. In Parkinson's disease DA deficiency causes an
increase in DA D2 receptors. The nicotinic acetylcholine receptor (nAChR) is the prototype for a superfamily of ligand gated ion channels (Corringer et al., 2000) that includes inhibitory [glycine (Gly), GABA_\text{\textalpha}, and GABA receptors] as well as excitatory receptors (nAChRs and 5-HT_3 receptors). These receptors have a pentameric structure, whereby the five subunits are arranged in a quasisymmetric distribution around a central pore (Unwin et al., 1988). Each subunit presents a large extracellular amino-terminal domain, folsess binding sites for ethanol (Crews et al., 1996). GABA, the major inhibitory neurotransmitter of the CNS is affected by even short-term exposure to ethanol and increases GABAergic function. Long-term ethanol exposure is associated with reduced GABA-benzodiazepine receptor (GBzR) levels and function (Lingford-Hughes et al., 2002). Ethanol enhances the activity of GABA, but reduces the excitatory effects of glutamate. These actions are the main reason that ethanol is often thought of as a depressant. GABA_\text{\textalpha} receptor is involved in ethanol’s acute and chronic effects (Mehta & Ticku, 1999; Buck & Finn, 2000; Cagett et al., 2003). Baclofen, agonist of GABA activates another type of GABA receptor (GABA_\text{B}), has recently been shown in a preliminary study to be effective in inducing abstinence from ethanol and reducing ethanol craving and consumption (Addolorato et al., 2002). Serotonin and dopamine are the major neurotransmitters involved in ethanol addiction in vivo (Tank, 1981). Serotonin produced and released from neurons that originate within discrete regions, or nuclei, in the brain (Cooper & Bloom, 1991). Along with other neurotransmitters, serotonin plays an important role in the brain process underlying ethanol abuse (David, 1999). Alterations in monoamines are observed in the striatum after chronic ethanol administration (Vasconcelos et al., 2004). DA is a neurotransmitter that has been implicated in various central neuronal degenerative disorders like Parkinson's disease and
behavioural diseases like Schizophrenia. DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised. DA interacts with specific membrane receptors to produce its effects. These effects are terminated by re-uptake of DA into the presynaptic neuron by a DA transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). DA plays an important role both centrally and peripherally. Nonetheless, the mesolimbic DA system has been shown to play a role in the rewarding effects of ethanol. The recent identification of five DA receptor subtypes provides a basis for understanding DA's central and peripheral actions. Stimulation of the DA D₁ receptor gives rise to increased production of cAMP. DA D₂ receptors inhibit cAMP production, but activate the inositol phosphate second messenger system. Impairment of central DA neurotransmission causes muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Ethanol enhanced 5-HT₃A receptor function, but had no effect on mouse 5-HT₃A/B receptor mediated currents (Hayrapetyan et al., 2005). Ethanol administration activates the HPA axis (Ellis, 1966, Rivier et al., 1984, Rivier & Vale, 1988; Thiagarajan et al., 1989; Rivier, 1996; Rivier & Lee, 1996; Ogilvie et al., 1997). Acetaldehyde formed in brain is able to activate the HPA axis at a central level (Hiroshi et al., 2001). Aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Amir, 1977). Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton et al., 1977), which may be one of the important factors in modifying ethanol-induced behaviour (Roberta et al., 2001). Ethanol is found to cause several biochemical changes in the NA, such as increased levels of tyrosine
hydroxylase, NMDA R1 and Glutamate R1 receptor subunits and decreased levels of subunit α1 of the GABA<sub>A</sub> receptor complex (Ortiz et al., 1995).

**Dopamine**

Dopamine (DA) exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the DA transporter (DAT). DA containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Carlsson, 1993; Lookingland et al., 1995; Creese et al., 1997; Tarazi et al., 1996, 2001). Dopaminergic system is organized into four major subsystems (i) the nigrostriatal system involving neurons projecting from the substantia nigra - the major DA system in the brain as it accounts for about 70% of the total DA in the brain, and its degeneration makes a major contribution to the pathophysiology of Parkinson’s disease; (ii) the mesolimbic system that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex, all of which are considered components of the limbic system and so are of particular interest for the patho-physiology of idiopathic psychiatric disorders; (iii) mesocortical pathway arising from the arcuate and other nuclei of the hypothalamus the mesocortical system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the tuberinfundibular pathway, which is a neuroendocrinological and ending in the median eminence of the inferior hypothalamus. DA released in this system
exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, this chemical messenger is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain. The DA transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The DA content which is about 70 to 80% in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine, are able to block the action of the DA transporter, thereby sustaining the presence of DA in the synaptic cleft and its action on DA receptors. Part of the DA is inactivated by conversion to inactive compounds by metabolic enzymes, which are present both intra- and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase and COMT are responsible for the metabolism of DA. DA after reuptake may intraneuronally be deaminated by MAO to give 3, 4-dihydroxyphenyl acetaldehyde (DOPAL), which subsequently is converted to 3, 4-dihydroxyphenylacetic acid (DOPAC) by ALDH. DOPAC is then methylated by COMT to give homovanillic acid (HVA).

**DA receptors**

DA mediates its actions via membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA-enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. DA receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the
five DA receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intracytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Carlsson, 1993; Tarazi et al., 1996; Creese et al., 1997). In their putative transmembrane domains, the DA D1 and D3 receptors are 79% identical to each other, while they are only 40–45% identical to the DA D2, D3, and D4 receptors. Conversely, the DA D2, D3, and D4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane-spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D1, the C-terminus in DA D2 like receptors. The DA receptors are glycosylated in their N-terminal domains. DA D1 like subtypes has potential glycosylation sites in their first extra cytoplasmic loop.

**DA receptor classification**

DA receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic-AMP (cAMP) (Calne, 1979; Schwartz et al., 1992; Civelli et al., 1993; Jackson et al., 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. DA D1 like receptors are characterized initially as mediating the stimulation of cAMP
production. DA D₂ like receptors inhibit the production of cAMP. This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin \textit{in vivo} and \textit{in vitro} in a cAMP-independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D₃, D₄ and D₅, with different anatomical localization from traditional DA D₁ or DA D₂ receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D₁-like family which includes DA D₁ and D₅ receptors. The DA D₂-like family includes DA D₂, D₃ and D₄ receptors (Shen \textit{et al.}, 1993).

\textbf{DA D₁-like family}

The DA D₁-like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli \textit{et al.}, 1993). They are classified into DA D₁ and D₅. The DA D₁-like receptors have short third intracellular loops and long carboxy terminal tails. The DA D₁ receptor is the most abundant DA receptor in the central nervous system. In the DA D₁ and DA D₅ receptors third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. The small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors can be probably related to the third cytoplasmic loop and the carboxy terminal tail (Gingrich \textit{et al.}, 1993; Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter
(27 amino acids) in the D₁ receptor than in the D₅ receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DA D₅ receptor (Marc et al., 1998).

**DA D₁ receptor**

DA D₁ receptors are found at high levels in the typical DA regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. DA D₁ receptor seems to mediate important actions of DA to control movement, cognitive function and cardiovascular function. The DA D₁ receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP₃) and diacylglycerol via the activation of phospholipase C (Sibley et al., 1990; Monsma et al., 1990). DA D₁ receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DA D₁ receptors mRNA is colocalized in striatal neurons of the basal ganglia with mRNA for DA receptor phospho protein (DARPP-32; KD) which is a DA and cyclic-AMP-regulated phosphoprotein. DA receptor phosphoprotein contributes to the actions of D₁ receptor (Hemmings & Greengard, 1986; Greengard et al., 1987).

**DA D₅ receptors**

The gene encoding the human DA D₅ protein is located at the short arm of chromosome 4, the same region where the Huntington disease gene has been located (Gusella, 1989). The DA D₅ receptor gene is intronless and encodes a protein that extends for 477 amino acids (George et al., 1991). This protein has an overall 50% homology with DA D₁ receptor and 80% if only the seven transmembrane segments are considered. Two DA D₅ receptor pseudogenes
having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Allen et al., 1991). DA D5 receptors, like DA D1 receptors, appear to interact with G-proteins and can stimulate adenylyl cyclase, with relatively high affinity for DA and DA D1-selective agonists (George et al., 1991). DA D5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli et al., 1992). It is involved in the thalamic processing of painful stimuli (Basbaum et al., 1979).

**DA D2-like family**

The dopamine D2 receptor is one of at least five physiologically distinct dopamine receptors (D1, D2, D3, D4 and D5) found on the synaptic membranes of neurons in the brain (Sibley & Monsma, 1990). DA D2-like receptors are characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. DA D2-like receptors belong to the G-protein coupled receptors and have 400 amino acid residues. The function of sugar moieties is unclear (Marc et al., 1998; Sibley, 1999). The unique feature of DA D2-like receptors family is that they possess a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc et al., 1998). It is generally believed that the membrane enclosed part of the amino-acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino-acid residues. Between the different DA receptors, the third loop also displays the
greatest variability in amino-acid sequence. This may have consequences for their respective second messenger systems. The DA D2-like receptors are coupled to G-protein and inhibit the formation of cyclic AMP. The DA D2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

DA D2 receptors

The DA D2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D2 receptor has seven transmembrane segments, but in contrast to DA D1-like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. The gene encoding this DA D2 receptor was found to reside on q22-q23 of human chromosome 11 (Makar et al., 1989). The DA D2 receptor was the first receptor to be cloned (Chrisre et al., 1988). The DA D2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum et al., 1986) activation of potassium channels and potentiation of arachidonic acid release (Axelrod et al., 1991). The DA D2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz et al., 1991). The DA D2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as DA D2S and DA D2L (Seeburg et al., 1989; Marc et al., 1998). DA D2 receptor isoforms (DA D2L and DA D2S) vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D2 receptor peptide chain. Both variants share the same distribution pattern; with the shorter form less abundantly transcribed in addition they appear to differ in their mode of regulation (Marc et al., 1998). Because this loop seems
to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D₂ receptor isoforms. The two isoforms derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D₂ receptor pre-mRNA (Schwartz et al., 1989). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D₂-selective agents, and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley et al., 1994). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to DA treatment is reported: DA induces the up-regulation of DA D₂L isoform of DA D₂ receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc et al., 1998; Sibley, 1999). However, the DA D₂S receptor isoform displayed higher affinity than the DA D₂L in this effect (Seeburg et al., 1989; Marc et al., 1998). The isoforms of DA D₂ mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk⁻ fibroblasts. Protein kinase C (PKC), however, differentially modulates DA D₂S and DA D₂L-activated transmembrane signalling in this system with a selective inhibitory effect on the DA D₂S-mediated response.

DA D₃ receptors

The gene encoding this receptor resides on chromosome 3 (Giros et al., 1990). DA D₃ mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz et al., 1991). DA D₃ receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz et al., 1990). The DA D₃
receptors bear close structural and pharmacological similarities to the DA D2 receptors. Distribution of DA D3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia (Marc et al., 1998). D3 receptor mRNA has also been found in neurons of the cerebellum, which may regulate eye-movements (Levesque et al., 1992). The structural similarity with DA D2 receptor raises the possibility that DA D3 receptor may also inhibit adenylyl cyclase activity in its normal cellular setting. More recent studies reported that DA D3 receptors might mediate positive regulatory influences of DA on production of the peptide neurotensin (Levesque et al., 1995; Marc et al., 1998). The status of the DA D3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism (Schwartz et al., 1990; Sokoloff et al., 1992; Marc et al., 1998).

DA D4 receptors

The gene encoding the human DA D4 protein is located at the tip of the short arm of chromosome 11 (Civelli et al., 1992; Marc et al., 1998). DA D4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes (Civelli et al., 1994). DA D4 receptor gene contains four introns and encodes a 387 amino acid protein (Van et al., 1991). The overall homology of the DA D4 receptor to the DA D2 and D3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. In humans, DA D4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the
third cytoplasmic domain (Marc et al., 1998). These are called the DA D₄ alleles, which are represented as DA D₄₂, D₄₄ and D₄₇. These may contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind, 1994). The stimulation of DA D₄ receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff et al., 1994; Marc et al., 1998).

**Effect of ethanol on brain DA receptors**

Since the first report by Blum et al., (1990) suggesting an association of DA D₂ receptor gene and ethanol addiction, the possible role of DA D₂ receptor locus in the etiology of ethanol addiction has been the focus of considerable attention (Noble, 2000). The brain of ethanol addicts seems to contain abnormalities that reduce the effectiveness of the dopaminergic system. Chronic ethanol consumption has been associated with an increased DA turnover rate and decreased DA uptake (Mash et al., 1996). Striatal dopamine deficit is correlated with ethanol craving. Dopaminergic D₂ receptor mechanisms are involved in the biology of ethanol dependence in man (Hietala et al., 1994). Human genetic studies suggest that an association exists between ethanol addiction and both the DA D₂ receptor and the DA transporter. This is supported by brain imaging studies that have reported alterations in both DA D₂ receptor and DA transporter densities in the brain of ethanol addicts (Repo et al., 1999). Reward-related impulsiveness may constitute a risk factor for ethanol dependence and that this core temperament could be partly mediated by the DA D₂ gene (Limosin et al., 2003). Continuous chronic or repeated deprivations increase binding sites of D₁.
and D₂ receptors in specific regions of the extended amygdala (EA) with greater sensitivity in the anterior regions (Sari et al., 2006).

**DA receptor gene expression and ethanol**

The genes encoding DA receptor subtypes have received considerable attention for the past several years as a potential candidate that may affect susceptibility to addictive disorder, including ethanol addiction (Lee et al., 2002). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. DA D₁-like receptors genes do not contain introns in their coding regions, a characteristic shared with most G protein-coupled receptors. The genes encoding the DA D₂-like receptors are interrupted by introns (Marc et al., 1998). Furthermore, most of the introns in the DA D₂-like receptor genes are located in similar positions. The DA D₂ receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer et al., 1989). ALDH genes are involved in dopamine metabolism and they interact with the DA D₁ receptor genes in alcohol dependence (Huang et al., 2004). The constitutive expression of D₂ receptor short isoform also reduced the tumor cell growth rate (Sarkar et al., 2005). Dopamine acts through G-protein-coupled D₂ receptors to affect the amount of intracellular cyclic AMP (Hayes et al., 1992). The DA D₁ receptor gene, which lacks any introns, encodes a protein that extends for 446 amino acids (Caron et al., 1991). In humans DA D₁ receptor gene has been localized to chromosome 5 (Kennedy et al., 1990). Dopamine receptor genes responsive to alcohol exposure encode proteins which
are involved in growth hormone (GH) release and its expression is altered by chronic alcohol intake (Gerhard et al., 2006). DA D₂ receptors activation inhibits norepinephrine gene expression and release in the arcuate nucleus and peripheral nerves (Carey et al., 1983; Pelletier et al., 1991). D₂ receptor gene A1 allele shows a significantly higher prevalence in ethanol users compared with nonusers (Comings et al., 1994; Noble, 1996).

**Serotonin**

Serotonin (5-HT) is widely distributed in both the animal and the plant kingdoms and is found in such diverse locations as tunicates, molluscs, arthropods, fruits, nuts and venoms (Erspamer, 1996). The enormous range of this single brain chemical system may reflect the vast distribution of its fibers in brain, from a small group of large multipolar neurons. Serotonin is synthesized and released from neurons that originate within discrete regions, or nuclei, in the brain (Cooper & Bloom, 1991). 5-HT may be tied to the evolution of life itself, particularly through the role of tryptophan, its precursor molecule. Tryptophan is an indole-based, essential amino acid, which is unique in its light absorbing properties. In plants, tryptophan-based compounds capture light energy for use in metabolism of glucose, the generation of oxygen and reduced cofactors. Tryptophan, oxygen and reduced cofactors combine to form 5-HT. 5-HT-like molecules direct the growth of light-capturing structures towards the source of light. In plants, tryptophan produces receptor proteins which harness light and thus produce biologically important molecules (Josefsson & Rask, 1997). Chlorophyll, for example, captures light because it contains tryptophan, and then generates ATP, reduced cofactors (NADH), and oxygen. This entire process is blocked if tryptophan is substituted with another amino acid (Mogi et al., 1989).
Serotonin has effects on other neurotransmitter systems. Ascending serotonergic systems from the median and dorsal raphe innervate areas of the brain rich in DA neurons, where they regulate the firing rate and release of DA. Liu et al., (1992) have shown that serotonin, through regional effects on either raphe glia or mesencephalic glia, will promote nerve growth factors affecting maturity of serotonergic neurons. 5-HT is an endogenous amine involved in diverse biologic processes within the central and peripheral nervous system and the cardiovascular and gastrointestinal and respiratory systems (Hindle, 1994). It is reported that there is a hypothalamic serotonergic receptor functional regulation mediated through 5-HT3C receptor during pancreatic regeneration (Mohanan et al., 2005 a, b). Jackson & Paulose (1999) reported a decrease in brain 5-HT content during diabetes. 5-HT has been implicated more in behaviour, physiological mechanisms, and disease processes than any other brain neurotransmitter. This diversity of actions is made possible because of the existence of specific 5-HT cell surface receptor subtypes and their coupling to distinct intracellular messenger systems or ion channels (Hoyer et al., 1994). Serotonin through 5-HT2 receptor caused a dose-dependent increase in DNA synthesis in primary cultures of rat hepatocytes (Sudha & Paulose, 1997). The synthesis and degradation of 5-HT is a very active process and it has been estimated that the total body pool of 5-HT is replaced every 24 hours. The synthesis of 5-HT occurs primarily by enzymatic hydroxylation of the benzene ring of tryptophan to form 5-hydroxytryptophan (5-HTP) and then through decarboxylation of the terminal carbon group of 5-HTP to form 5-HT. Once inside the cells, 5-HT is degraded by monoamine oxidase to form an aldehyde, which is then hydrolysed by ALDH to form 5-HIAA, the principal metabolite excreted in urine. The neurons form a collection of clustered cells termed the
raphe nuclei, located on the exact midline of the brainstem. Serotonergic fibers interact in complex ways with a variety of cell types—neurons, glial cells, endothelial cells, ependymal cells and others by binding to at least 14 distinct receptor proteins. Furthermore, 5-HT neurons are one of the first brainstem neurons to emerge during early development of the brain and spinal cord present by the sixth week of gestation in humans. In rats, 5-HT neurons in the brainstem raphe are among the first neurons to differentiate in the brain and play a key role in regulating neurogenesis (Kligman & Marshak, 1985). The 5-HT neurons are the first neuronal system to innervate the primordial cortical plate. During development, 5-HT fibers arrive at the cortical plate during the peak period of mitosis and maturation (Dori et al., 1996). Lauder & Krebs (1978) reported that para-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, retarded neuronal maturation. Since then, many other workers have shown a role for 5-HT in neuronal differentiation (Marois & Croll, 1992; Rodriguez, 1994).

**5-HT receptor classification**

5-HT receptors can be classified into seven classes from 5-HT_1_ to 5-HT_7_, based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms of receptors (Bradley et al., 1986; Zifa & Fillion, 1992; Peroutka, 1993). All 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a seven transmembrane domain structure except 5-HT_3_ receptor, which forms a ligand-gated ion channel.
5-HT\textsubscript{1} Receptor

Five 5-HT\textsubscript{1} receptor subtypes have been recognised, 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, 5-HT\textsubscript{1D}, 5-HT\textsubscript{1E} and 5-HT\textsubscript{1F}. All are seven transmembrane, G-protein coupled receptors encoded by intronless genes, of between 365 and 422 amino acids with an overall sequence homology of 40\%. 5-HT\textsubscript{1A} receptor subtype which is located on human chromosome 5q11 is widely distributed in the CNS, particularly hippocampus (Hoyer et al., 1994). The 5-HT\textsubscript{1B} receptor is located on human chromosome 6q13 and is concentrated in the basal ganglia, striatum and frontal cortex. The receptor is negatively coupled to adenylyl cyclase. The 5-HT\textsubscript{1D} receptor has 63\% overall structural homology to 5-HT\textsubscript{1B} receptor and 77\% amino acid sequence homology in the seven transmebrane domains. The receptor is located on human gene 1p36.3-p34.3 and is negatively linked to adenylyl cyclase. The 5-HT\textsubscript{1E} receptor was first characterized in man as a $[^3H]5$-HT binding site in the presence of 5-carboxyamidotryptamine (5-CT) to block binding to the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1D} receptors. It is reported that the brain 5-HT through 5-HT\textsubscript{1A} receptor has a functional role in the pancreatic regeneration through the sympathetic regulation (Mohanan et al., 2005). Human brain binding studies have reported that 5-HT\textsubscript{1E} receptors are concentrated in the caudate putamen with lower levels in the amygdala, frontal cortex and globus pallidus (Hoyer et al., 1994). This is consistent with the observed distribution of 5-HT\textsubscript{1C} mRNA (Hoyer et al., 1994). The receptor has been mapped to human chromosome 6q14-q15, is negatively linked to adenylyl cyclase and consists of a 365 amino acid protein with seven transmembrane domains. 5-HT\textsubscript{1F} receptor subtype is closely related to the 5-HT\textsubscript{1E} receptor with 70\% sequence homology.
across the 7 transmembrane domains. mRNA coding for the receptor is concentrated in the dorsal raphe, hippocampus and cortex of the rat and also in the striatum, thalamus and hypothalamus of the mouse (Hoyer et al., 1994).

5-HT$_2$ Receptor

The 5-HT$_2$ receptor family consists of three subtypes namely 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. All three are single protein molecules of 458-471 amino acids with an overall homology of approximately 50% rising to between 70-80% in the seven transmembrane domains. 5-HT$_{2A}$ receptor previously termed as 5HT$_2$ receptor is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues. It mediates contractile responses of vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue (Hoyer et al., 1994). 5-HT$_{2C}$ was previously termed as 5-HT$_{1C}$ before its structural similarity to the 5-HT$_2$ family members was recognized. All three are thought to be linked to the phosphoinositol hydrolysis signal transduction system via the $\alpha$ subunit of Gq protein. It is reported the involvement of serotonin, S$_2$ receptors in the DNA synthesis of primary culture of rat hepatocytes (Balasubramanian & Paulose, 1998). In human pulmonary artery endothelial cells, 5-HT$_{2C}$ receptor stimulation causes intracellular calcium release via a mechanism independent of phosphatidylinositol hydrolysis (Hagan et al., 1995). The 5-HT$_{2B}$ receptor located on chromosome 2q36-2q37.1 mediates contraction of the rat stomach fundus and endothelium dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery, via nitric oxide release.
(Choi & Maroteaux, 1996). $5\text{-HT}_{2B}$ receptor mRNA has been detected throughout the mouse, rat and guinea pig colon and small intestine. $5\text{-HT}_{2C}$ specific antibodies have shown the presence of the receptor protein in the choroid plexus, in higher density and at a lower density in the cerebral cortex, hippocampus, striatum, and substantia nigra of rat and a similar distribution in man. The receptor has been mapped to human chromosome Xq24. No splice variants have been reported but the receptor is capable of post translational modification whereby adenosine residues can be represented as guanosine in the second loop to yield 4 variants.

**5-HT$_3$ Receptor**

Unlike other 5-HT receptors, 5-HT$_3$ receptor subunits form a pentameric cation channel that is selectively permeable to Na$^+$, K$^+$ and Ca$^{2+}$ ions causing depolarisation. The 5-HT$_3$ receptor is a member of a superfamily of ligand-gated ion channels, which includes the muscle and neuronal nAChR, the gly and GABA$_A$ receptor (Unwin, 1993; Karlin & Akabas, 1995; Ortells & Lunt, 1995). The 5-HT$_3$ receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa and nuclei of the lower brainstem. It is also found in higher brain areas such as the cortex, hippocampus, amygdala and medial habenula, but at lower densities. Like the other members of the gene superfamily, the 5-HT$_3$ receptor exhibits a large degree of sequence similarity and thus presumably structural homology with the AChR (Maricq et al., 1991).
5-HT\textsubscript{4} Receptor

The receptor is functionally coupled to the G protein. Receptor binding studies have established that the 5-HT\textsubscript{4} receptor is highly concentrated in areas of the rat brain associated with DA function such as the striatum, basal ganglia and nucleus accumbens. These receptors are also located on GABAergic or cholinergic interneurons and/or on GABAergic projections to the substantia nigra (Patel \textit{et al.}, 1995).

5-HT\textsubscript{5} Receptor

5-HT\textsubscript{5} receptors have thus been classified as 5-HT\textsubscript{5A} and 5-HT\textsubscript{5B} and their mRNAs have been located in man (Grailhe \textit{et al.}, 1994). Two 5-HT receptors identified from rat cDNA and cloned were found to have 88\% overall sequence homology, yet were not closely related to any other 5-HT receptor family (Erlander \textit{et al.}, 1993). In cells expressing the cloned rat 5-HT\textsubscript{5A} site, the receptor was negatively linked to adenylyl cyclase and may act as terminal autoreceptors in the mouse frontal cortex (Wisden \textit{et al.}, 1993).

5-HT\textsubscript{6} Receptor

Rat and human 5-HT\textsubscript{6} mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs studied (Kohen \textit{et al.}, 1996). Like the 5-HT\textsubscript{5} receptor, the 5-HT\textsubscript{6} receptor has been cloned from rat cDNA based on its homology to previously cloned G protein coupled receptors. The rat receptor consists of 438 amino acids with seven transmembrane domains and is positively coupled to
adenylyl cyclase via the Gs G protein. The human gene has been cloned and has 89% sequence homology with its rat equivalent and is coupled to adenylyl cyclase (Kohen et al., 1996).

5-HT₇ Receptor

5-HT₇ receptor has been cloned from rat, mouse, guinea pig and human cDNA and is located on human chromosome 10q23.3-q24.4. Despite a high degree of interspecies homology (95%) the receptor has low homology (<40%) with other 5-HT receptor subtypes.

Effect of ethanol on brain 5-HT receptors

The serotonergic system, because of very diffuse projections throughout the central nervous system, has been implicated in numerous functions including nociception, analgesia, and autonomic regulation (Jolas & Aghajanian, 1997). 5-HT systems contribute to the discriminative properties of ethanol in animals and humans. Ethanol facilitates that activity of 5-HT₁B, 5-HT₂C, 5-HT₃ receptors, and it shares discriminative stimulus properties with drugs acting at these sites (Grant et al., 1995 & 1997). Serotonergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic system (Koob & Weiss 1992). Levels of brain 5-HT receptor are inversely related to ethanol consumption (Pandey et al., 1992; LeMarquand et al., 1994; Himei et al., 2000). The m-chlorophenylpiperazine (m-CPP) is a serotonin agonist which has been reported to elicit craving for ethanol (Benkelfat et al., 1991; Krystal et al., 1994). Ethanol is a positive modulator at the 5-HT₃ receptor, which has been implicated in ethanol drinking, anxiety and aggression (McKenzie et al., 2005; Hayrapetyan et al., 2005) but 5-HT₁D receptor plays little role in the
pathophysiology of ethanol addiction (Bavanisha et al., 2005). Acute ethanol exposure enhances the electrical signals generated by the 5-HT_3 receptor. When activated by serotonin binding, the 5-HT_3 receptor rapidly increases neuron activity by generating electrical signals (Lovingier & Peoples, 1993; Lovingier & Zhou, 1994). Chronic ethanol treatment may decrease serotonergic neurotransmission in selective brain regions. Serotonin receptor polymorphism reflects the pathogenesis of ethanol addiction (Yoshihara et al., 2000).

5-HT receptor gene expression and ethanol

Ethanol and drugs of abuse indirectly induce the expression of a number of genes, which, in the context of protein synthesis, activate several biochemical pathways in brain neurons (German et al., 1999). A common insertion-deletion polymorphism in the promoter region for the serotonin transporter gene alters in vitro gene transcription, (Lesch et al., 1996) in vitro transporter availability (Stoltenberg et al., 2002) and in vivo serotonin transporter density (Heinz et al., 2001). There have been several associations of this polymorphism to behaviours and traits that relate to excessive alcohol intake and serotonin transporter gene promoter variation have been associated with alcohol consumption in human and animal populations (Christina et al., 2004). 5-HT_1D receptor mRNA is found in the rat brain, predominantly in the caudate putamen, nucleus accumbens, hippocampus, cortex, dorsal raphe and locus coeruleus (Hoyer et al., 1994). Genetic variability in the 5-HT_2A receptor is involved in the development of ethanol dependence (Nakamura et al., 1999). The human 5-HT_1B receptor, encoded by the 5-HT_1B gene, is a presynaptic serotonin autoreceptor that plays a role in regulating serotonin synthesis and release. 5-HT_1B receptor is associated with alcohol dependence (Sun et al., 2002). Hofmann et al., (2002) reported that
prenatal ethanol exposure alters 5-HT_{1A} and 5-HT_{2A} receptor function in adulthood. 5-HT_{6} receptor mutant mice demonstrated reduced responses to the sedative effects of ethanol (Bonasera et al., 2006)

**Brain neurotransmitters and ethanol**

Brain is the major target for the actions of ethanol, and heavy ethanol consumption has long been associated with the brain damage. Brain neurotransmitters through their receptors play an important role in governing the cellular activities. The acute and chronic ethanol ingestion has been shown to induce significant changes in neurotransmitter systems (Nevo & Hamon, 1995). Ethanol can pass through cell walls and is distributed throughout the water content of tissues and cells. In its circulation through the body and reaches the brain. Multiple neurotransmitter systems play a role in mediating the behavioural effects of ethanol that have been linked to its abuse and dependence (Koob & Weiss, 1992). At the neurochemical level, the moderate consumption of ethanol selectively affects the function of GABA, glutamatergic, serotonergic, dopaminergic, cholinergic, and opioid neuronal systems. Ethanol can affect these systems directly, and/or the interactions between and among these systems become important in the expression of ethanol's actions (Eckardt et al., 1998).

**DA and 5-HT**

Ethanol is similar to other abused substances in that it increases nucleus accumbens (NAcc) DA release. Furthermore, innate differences in central dopaminergic neurotransmission have been linked to high levels of ethanol drinking in selectively bred rodent lines (Li, 2000). Alterations of DA activity within the Extended Amygdala (EA) after chronic exposure to ethanol or
substances of abuse are considered a major mechanism for the development of ethanol addiction (Sari et al., 2006). Neuronal DA receptors are widely distributed in the central (Kebabian et al., 1979) and the peripheral nervous system at different levels. On the other hand, DA and 5-HT interact antagonistically in the dorsal striatum to control motor activity. Serotonin is one of the major neurotransmitter involved in ethanol addiction in vivo (Tank et al., 1981). Along with other neurotransmitters serotonin play an important role in the brain process underlying ethanol abuse (David, 1999). 5-HT2 agonists, as well as serotonin reuptake inhibitors, have been found to substitute for ethanol in drug discrimination tests (Signs & Schechter, 1988; Maurel et al., 1997). 5-HT3 activity is probably responsible for the nausea with excessive ethanol consumption (Wilde & Markham 1996). It is also likely to partially account for increased dopamine release as antagonists have been shown to block ethanol induced dopamine release (Carboni et al., 1989; Badawy et al., 1995). Serotonin can alter dopaminergic signal transmission in several ways. For example, by interacting with the 5-HT2 receptor, serotonin stimulates the activity of dopaminergic neurons in a brain region called the VTA, thereby enhancing an ethanol-induced increase in the activity of these neurons (Brodie et al., 1995). Serotonin also interacts with dopaminergic signal transmission through the 5-HT3 receptor, which helps control dopamine release in the areas reached by VTA neurons, most notably the nucleus accumbens. Serotonin release in these brain regions can stimulate dopamine release, presumably by activating 5-HT3 receptors located on the endings of dopaminergic neurons (Grant, 1995). 5-HT depletion resulted in increased ethanol consumption in animals and humans (Melchior & Tabakoff, 1986; Higley et al., 1996; Jankowska et al., 1994).
Dopamine's precise role in the development of ethanol addiction remains unclear (Rassnick et al., 1993; Di Chiara, 1995).

**Acetylcholine**

Acetylcholine is the neurotransmitter of the parasympathetic system. Acetylcholine, acting on presynaptic nAChRs, modulates the release of neurotransmitters in the brain (Centeno et al., 2006). Cholinergic receptors are classified as ionotropic nicotinic receptor and metabotropic muscarinic receptor. Muscarinic receptors are classified as M₁, M₂, M₃, M₄ and M₅. They are G-protein coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions that interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects. Ethanol enhances the activity of alpha4beta2 neuronal nicotinic acetylcholine receptor and support the possibility that a polymorphism of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) modulates enhancement of nicotinic receptor function by ethanol (Kim et al., 2004). Increased muscarinic M₁ and M₃ receptor activity at the time of pancreatic regeneration is reported to facilitate insulin secretion and beta cell proliferation (Renuka et al., 2005). The striatum receives converging glutamatergic input from cortex and thalamus as well as dopaminergic input from the substantia nigra. Integration of these extrinsic inputs is modulated by the intrinsic actions of acetylcholine (ACh). Striatal ACh is supplied by large-sized cholinergic interneurons the functions of which are still not well characterized (Kawaguchi, 1993). At the cellular level, both striatal ACh and DA are potent neuromodulators that can affect activity-dependent changes in synaptic efficacy and may contribute to motor or habit learning (Wickens et al., 1996; Calabresi et al., 1992, 2000; Tang et al., 2001).
The stimulatory, rewarding, and DA enhancing effects of ethanol involve central nAChR, especially those located in the ventral tegmental area (VTA) (Jerlhag et al., 2006). AChRs are expressed at high levels in striatum. Muscarinic acetylcholine receptors are expressed both presynaptically and postsynaptically in striatum, and one of their actions is to decrease glutamatergic synaptic transmission (Malenka & Kocsis, 1988; Hersch et al., 1994). nAChRs are expressed on dopaminergic terminals in the dorsal striatum (Clarke & Pert, 1985). Acute activation of these receptors stimulates DA release from striatal synaptosomes and in striatal slice preparations (Giorgieff et al., 1976; Kulak et al., 1997; Wonnacott et al., 2000). The cholinergic system is yet another target for the actions of ethanol (Narahashi et al., 1999) and has been found to act as a co-agonist with acetylcholine at the nAChRs, as well as to potentiate the effect of nicotine at this receptor, both of which ultimately results in an increase in mesolimbic dopamine (Soderpalm et al., 2000).

**Epinephrine and Norepinephrine**

The sensitivity of noradrenergic systems to ethanol effects varies among brain regions (Tabakoff & Hoffman, 1996). Ethanol consumption increases central and peripheral levels of epinephrine (EPI) and norepinephrine (NE), which contributes to the stimulatory affects of ethanol, particularly in the ascending arm of the blood ethanol curve (Pohorecky, 1982), brain levels of norepinephrine have been shown to increase up to three-fold (Wang et al., 1993). It is reported a significant increase in the NE content in the brainstem during diabetes (Jackson et al., 1997, 1999). The locus coeruleus (LC) contains the cell bodies for the brain dorsal noradrenergic system (Grzanna & Molliver, 1980). LC basal activity and activation are reduced by ethanol, an action that may contribute
to sedative effects of ethanol (Aston-Jones et al., 1982; Shefner & Tabakoff, 1985). These elevations occur primarily due to increased release and decreased clearance, rather than increases in synthesis (Howes et al., 1986). A consequence of this is eventual depletion of epinephrine and norepinephrine in the adrenals after 4 days of ethanol intoxication (Adams & Hirst, 1984). This decrease contributes to the CNS depression that occurs with prolonged drinking. Changes in the levels of DA, 5-HT, NE, and their metabolites in several regions of the rodent brain, many of them involved ethanol treatment for a short period of time and withdrawal (Yan, 1999; Yoshimoto et al., 2000). Ethanol activates the norepinephrine system in the limbic circuitry through an intercellular cascade that includes serotonin, opioid peptides and dopamine. Ethanol may also act directly through the production of neuroamines that interact with opioid receptors or with dopaminergic systems (Alvaksinen et al., 1984; Blum & Kozlowski, 1990). Central alpha1-adrenergic receptors have a functional role in the pancreatic regeneration mediated through the sympathetic pathway (Ani et al., 2006). Ethanol has a variety of effects on neuroendocrine function and there is a great deal of interest in investigating the effects of ethanol on the HPA axis (Rivier et al., 1984). The by-products of ethanol metabolism include acetaldehyde, which may have an inhibitory effect on the adrenergic receptors. Increased cyclic adenosine monophosphate in neurons with long term ethanol exposure may increase norepinephrine receptor sensitivity and norepinephrine turnover (Keltner et al., 1998). α-Adrenergic stimulation attenuates ethanol intoxication, whereas β-adrenergic blockade enhances intoxication (Alkana et al., 1976 & 1977).
Gamma aminobutyric acid and glutamate

GABA system - the body's primary inhibitory pathway (Meldrum, 1982), ethanol potentiates GABA's activity (Suzdak et al., 1986) acting through GABA_A receptors. It likely has a biphasic effect on behaviour, with lower doses inhibiting inhibitory GABA interneurons on dopamine receptors in the VTA thus causing dopamine induced stimulation and euphoria, and higher doses producing widespread inhibition of CNS activity, thus overriding the stimulant effects (Kalivas et al., 1990; Grobin et al., 1998). This is likely one of the major mechanisms through which it produces its sedative-hypnotic and anxiolytic actions. One of the most powerful actions of ethanol is to reduce the overall level of brain activity by a combination of effects on two key neurotransmitters, GABA and glutamate. Ethanol reduces the excitatory effects of glutamate. The n-Methyl-d-Aspartate (NMDA) receptor is one of three types of glutamate receptors - the body's primary excitatory neurotransmitter. It is named for NMDA, its synthetic, high-affinity ligand (Woodward, 2000), ethanol has been found to block the action of this receptor (Dildy & Leslie, 1989). The likely mechanism is by preventing glutamate's removal of a magnesium ion which blocks calcium influx into the cell (Collingridge & Bliss, 1995). This decreases the excitation of the cell, which, along with increased inhibition via GABA, results in the sedative-depressant effects of ethanol, particularly at higher doses. Chronic consumption of ethanol gradually makes the NMDA receptors hypersensitive to glutamate while desensitizing the GABAergic receptors.
Liver and ethanol

Ethanol effects on the human body and its health, the liver plays a particular important role (Yue et al., 2006) and the hepatic enzymatic systems involved in ethanol metabolism are ADH, ALDH and microsomal P4502E1 (CYP2E1) (Gemma et al., 2006). Acute ethanol intoxication may cause the changes of hepatic enzymes (Rakonczay et al., 2003; Yue, 2006). The intragastric administration of ethanol induced some morphological disturbances in the liver (Zimatkin et al., 1997). Ethanol metabolism causes oxidative stress (Rakonczay et al., 2003) and lipid peroxidation not only in liver but also in extra-hepatic tissues. Ethanol administration has been shown to cause oxidative degradation and depletion of hepatic mitochondrial DNA (mtDNA) (Abdellah et al., 2001). Chronic ethanol-induced decrease in the NAD dependant glycerol 3-phosphate dehydrogenase reaction was due to a decreased rate of NADH reoxidation in the liver (Manfred et al., 1998). In the rat liver, both mitochondrial and cytosolic ALDH are functional (Klyosov et al., 1996). Acetaldehyde, the first metabolite of ethanol, is produced in the liver following the first step of ethanol metabolism and is ten times more toxic than ethanol (Brien & Loomis, 1983). Acetaldehyde appears to mediate some of the behavioural & central neurotoxic effects of ethanol (Hunt, 1996).

Aldehyde dehydrogenase and ethanol

Mutations in ALDH genes cause inborn errors of metabolism such as the Sjogren-Larsson syndrome, type II hyperprolinaemia and gamma-hydroxybutyric aciduria and are likely to contribute to several complex diseases, including cancer and Alzheimer's disease. The ALDH gene products appear to be multifunctional
proteins, possessing both catalytic and non-catalytic properties (Vasiliou & Nebert, 2005). The aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Schlesinger et al., 1966; Sheppard et al., 1968; Amir, 1978; Socaransky et al., 1984). Also, this enzyme has been reported in mitochondria, microsomes and cytosol of rat liver (Tottmar et al., 1973). In some oriental populations with a lowered genetic activity of ALDH, high blood concentrations of acetaldehyde are produced following ethanol ingestion (Enomoto et al., 1991). As acetaldehyde is a highly toxic metabolite, it can cause adverse symptoms in susceptible individuals, including nausea, headache and palpitations (Enomoto et al., 1991). These individuals consume less ethanol than people who have normal activity of ALDH (Higuchi et al., 1992) and interestingly accumulation of acetaldehyde in blood following ethanol ingestion, due to a lower activity of ALDH, is believed to play a protective role against ethanol addiction (Harada et al., 1982). Although there are several reports that ethanol preference may correlate with ALDH activity more in the brain than in the liver (Amir, 1978; Socaransky et al., 1984), this mechanism is still relatively unknown (Minori et al., 2002). As acetaldehyde itself has many pharmacological actions (Brien & Loomis, 1983), it may act on the central nervous system (Kinoshita et al., 2001). Diadzin (Radix puerariae) an antidipsotropic agent could disturb an as-yet-undefined physiological pathway catalyzed by ALDH and alter the concentrations of some endogenous substrate(s) that regulate ethanol drinking behaviour. Rat liver mitochondrial preparations contained no detectable amounts of endogenous 5-HT, DA or any of their known metabolites (Wing, 1998). It is reported that oral treatment with the ALDH inhibitor disulfiram decreased ethanol preference (He et al., 1997). Early interest
in biogenic aldehydes, the metabolic intermediate of ethanol, interferes in some way with the oxidative metabolism of the brain. Epidemiological studies also have associated low MAO and/or high ALDH activities with high ethanol consumption (von Knorring, 1985), where differences in acetaldehyde elimination may contribute to ethanol preference. Brain plays an important regulatory role in hepatic functions (Lautt, 1983). The liver is richly innervated (Rogers & Hermann, 1983). Acetaldehyde produced from ethanol is metabolized quickly to acetate by ALDH. Brain monoamines and ALDH level together plays a decisive role in the ethanol addiction and ethanol addiction. 5-HT and its metabolic intermediates differentially regulate ethanol drinking. Serotonergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic reward system (Weiss, 1992). With long-term use, adolescent rats have shown massive neuronal loss in their cerebellum, basal forebrain, and neocortex (Spear, 2002). Endogenous DA plays a modulatory role on sympathetic nerve terminals through these receptors. ALDH genes involved in dopamine metabolism and ALDH genes interact with the DA D2 receptor gene and there is association between the DA D2 receptor gene and alcohol dependence (Huang et al., 2004). Strong ethanol preferences are associated with reduced serotonergic functions either directly or indirectly by increasing DA neurotransmission particularly in the ventral striatum (Koob, 1992). By speeding up the metabolism of ethanol to a toxic intermediate, acetaldehyde, or slowing down the conversion of acetaldehyde to acetate, genetic variants in the enzymes ADH or ALDH raise the level of acetaldehyde after drinking, causing symptoms that include flushing, nausea, and rapid heartbeat. The genes for these enzymes and the alleles, or gene variants that alter ethanol metabolism have been identified (Makimoto, 1998; Li, 2000). Ethanol metabolism is impaired by a
nonfunctional form of the enzyme aldehyde dehydrogenase (Wall & Ehlers, 1995) and consumption of even small amounts of ethanol may be severe (Goedde et al., 1992). Cyanamide (CY), a potent ALDH inhibitor in the liver, as well as in the brain (Hellstrom & Tottmar, 1982). The brain inhibition may alter the metabolism of biogenic amines by promoting the formation of condensation products or by increasing the levels of biogenic aldehydes. Extracellular concentration of both DA and 5-HT significantly decreased in the nucleus accumbens after acute intraperitoneal injection of acetaldehyde to rats (Ward et al., 1997).

**Nervous system and hepatic functions**

The autonomic nervous system influences many of the functions of the body, including those of cardiovascular system, kidneys, liver, pancreas, gastrointestinal tract and glands (Berthoud & Neuhuber, 2000). Brain plays an important regulatory role in hepatic functions (Lautt, 1983), signalling occurs between the liver and brain (Kerfoot et al., 2006). Normal brain functioning depends on several aspects of normal liver functioning; the liver supplies certain nutrients to the brain that the brain itself cannot produce. The liver also cleanses the blood of substances that could damage brain cells (i.e., neurotoxins). Liver dysfunction is associated with more extensive brain dysfunction in liver cirrhosis patients (Tarter et al., 1993). The autonomic nervous system directly innervates the hepatic parenchyma and has a role in metabolic control (Jungermann, & Stumpel, 1999). The autonomic nervous system plays a significant role in liver physiology and pathology (Stoyanova & Gulubova, 1998). After receiving information from afferent nerves, the hypothalamus sends signals to peripheral organs, including the liver, to keep homeostasis (Uyama et al., 2004). The liver
has innervations of nerves from the central nervous system. In the liver, the autonomic nervous system plays an important role (Stoyanova & Gulubova, 2000). The degree of liver dysfunction was associated with increasing severity of autonomic dysfunction (Frokjaer et al., 2006). Increased brain GABAergic neurotransmission is reported to regulate hepatic cell proliferation through the sympathetic stimulation (Biju et al., 2002). Hypothalamus controls liver functions by neural and neuroendocrine connections. The hypothalamus consists of three major areas: lateral, medial, and periventricular. Each area has some nuclei. There are two important nuclei and one area in the hypothalamus that send out the neural autonomic information to the peripheral organs. In addition to direct neural connections, the hypothalamus can affect metabolic functions by neuroendocrine connections: the hypothalamus-pancreas axis, the hypothalamus-adrenal axis, and the hypothalamus-pituitary axis (Uyama et al., 2004). Central nervous system modulates liver functions through the autonomic nervous system (Takayoshi, 2002). Miyajima et al., (2001) & Pozzi et al., (2001) reported that patients with liver cirrhosis have parasympathetic hypofunction and sympathetic hyperfunction. The hepatic parenchyma has been shown to have parasympathetic and sympathetic innervations (Nobin et al., 1978; Carobi & Magni et al., 1981; Rogers & Hermann, 1983). A selective 5-HT2 receptor agonist, 1-(2, 5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI) (Glennon, 1987) produced a tremendous increase in sympathetic nerve discharge (McCall et al., 1987). Enhanced GABA_B receptor was reported in neoplastic rat liver and hepatocyte cultures (Biju et al., 2002). Sympathetic nervous system inhibition increases hepatic progenitors (Oben et al., 2003). Mobilisation of 5-HT in intestine and its accumulation in liver and spleen tissues were observed at the initial periods after partial hepatectomy (Kulinskii et al., 1983). One subset of central nervous system
5-HT receptors (5-HT\textsubscript{1A}) can inhibit sympathetic nerve discharge while a second subset of receptors (5-HT\textsubscript{2}) can increase sympathetic nervous discharge (McCall & Harris, 1988). Hypothalamic GABA receptor subtypes was suggested to regulate hepatic cell proliferation (Biju et al., 2001). The central vagal connection with adrenergic and serotonergic innervations reaches the liver through the brainstem. The oxidation of fatty acids is the main energy source for the liver. Together with ethanol, isolated liver cells have a decreased oxidation of fatty acids. This is caused by the increased NADH: NAD\textsuperscript{+} ratio which can result in a decreased activity of the enzymes responsible for the \(\beta\)-oxidation (Forsell, 1981). The activity of the citric acid cycle decreases if the level of the cofactor NAD\textsuperscript{+} is too low. In that case, hydrogen equivalents from ethanol are used by the mitochondria instead of from the oxidation of fatty acids. This decrease of fatty acid oxidation may cause accumulation of fatty acids in the liver (Swanson & Sawchenko, 1980). These reports underlined the role of substantia nigra in modulating the outflow of both sympathetic and parasympathetic signals that ultimately reach the liver. Thyrotropin-releasing hormone (TRH) acts in the medulla, in particular in the left dorsal vagal complex, to induce stimulation of hepatic blood flow and hepatic proliferation, and protect against experimental liver injury through vagal and cholinergic pathways and neuropeptides such as beta-endorphin and bombesin in the brain modulate hepatic proliferation and bile secretion (Yoneda et al., 2001). TRH acts in the brain to increase hepatic cAMP through vagal-cholinergic and prostaglandin-dependent pathways, suggesting that central TRH modulates hepatic functions through cAMP-mediated signalling pathways (Yoneda et al., 2005). Hepatic encephalopathy is characterized by disturbances of motor and cognitive functions involving the basal ganglia (Sergeeva et al., 2005). CRF acts in the brain to decrease hepatic surface
perfusion and elevate portal pressure through central CRF$_2$ receptor and sympathetic-noradrenergic pathways (Yoneda et al., 2005).

**Nervous System and ALDH**

Rats consumed intoxicating quantities of ethanol when it was substituted for water (Lester, 1961; Senter & Sinclair, 1967; Everett & King, 1970; Falk et al., 1972; Freed, 1972; Meisch & Thompson, 1972; Ogata et al., 1972; Samson & Falk, 1974). ALDH is responsible not only for the metabolism of exogenous ethanol, but also for the oxidation of biogenic aldehydes in the central nervous system and in the periphery (Mostofa et al., 2003). It is known that a number of aldehydes occur in brain tissue (Blaschko et al., 1937; Pugh & Quastel, 1937) first presented evidence that aldehydes arise in brain tissue by the oxidative deamination of monoamines. Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton et al., 1977), which may be one of the important factors in modifying ethanol-induced behaviour. Three types of nerve endings are reported with in the liver. They are the sympathetic, parasympathetic and peptidergic nerves. The neurotransmitters found in these nerves are catecholamines, serotonin, acetylcholine, vasoactive intestinal polypeptides and cholecystokinin respectively. The nerve fibres enter the liver in association with the vascular supply. The peptidergic nerves are present in both the exocrine and endocrine tissues of this gland and there is considerable interspecies variability as to which part receives a greater proportion of these fibres. The nerve terminals end approximately 20-30nm from the endocrine cells thus implying that neurotransmitters affect several cells by diffusing through the extracellular space. The substantia nigra is one autonomic area in the central nervous system which plays an important role in controlling structure and activity of liver. Adaptation
in the ethanol metabolizing enzymes, explicitly of those enzymes responsible for the metabolism of ethanol's primary metabolite acetaldehyde is the critical factor on inclination towards ethanol preference (Ewing et al., 1974; Mizoi et al., 1979; Zeiner et al., 1979). Aldehyde dehydrogenase polymorphism results in change of effects of acetate and acetate-generated adenosine on the central nervous system and other organs during chronic ethanol consumption (Matsumoto, 1996). Hypothalamic origin of hypothyrodism and hypertension mediated through sympathetic stimulation was reported in pyridoxine deficient rats (Dakshinamurti et al., 1986; Paulose et al., 1988). The hypothalamic paraventricular nucleus has direct connections with the dorsal vagal complex mutation in the human fatty aldehyde dehydrogenase has been linked to a fetal neurological disorder called Sjogren-Larsson syndrome, and a change in ALDH activity has been observed in a number of tumors, including those of the liver, colon and breast. In short, ALDH is a vital enzyme involved with numerous processes of animal and plant health, most interestingly ALDH is involved in both biogenic amine metabolism (Berger & Weiner, 1977) and oxidation of biogenic aldehydes (Mostofa et al., 2003).

**Ethanol perfusion and ALDH**

The liver perfusion model has a great advantage over isolated and cultured hepatocytes techniques, as the hepatic architecture, polarity and the integrity of the cytoskeleton is maintained (Shattuck et al., 1993; Vom et al., 1995). Perfused liver appears to be a useful system for studies of enzymes like ADH - independent oxidation of alcohol (Cronholm et al., 1992). Desmoulin et al., (1987) reported that the perfusion of the liver with 70 mM ethanol not
change the adenine nucleotide levels, while the Pi content is decreased by 10%. More than 80% of ethanol was taken into the isolated rat liver and recovered as free acetate in the perfusate (Yamashita et al., 2001). The activity of ALDH in hepatic mitochondria was decreased by approximately 75% in carbon tetrachloride-intoxicated rat liver perfusion system (Yuki, et al., 1984). Glucose production decreased as a result of infusion of an amino acid mixture (Ali et al., 2000). It is reported that the ATP level significantly decreased at the beginning of the ethanol perfusion (Marie et al., 2004). Infusion of amino acid solutions caused an increase in glucose concentration was also found in the rat liver. Hepatic glucose release increased with increased amino acid uptake (Freetly et al., 1999). Liver infusion of glutamine or alanine alone increases glucose production by approximately 400% (Ali et al., 2006). Ethanol perfusion induces an increase in the in situ mitochondrial ATP/O ratio in the whole liver (Marie et al., 2002). The secretion of apoprotein B (ApoB) from the perfused liver was inhibited by noradrenaline or ATP (Yamauchi et al., 1998). A study on hepatic respiration and glycolysis in perfused rat livers showed ethanol decreased the rate of lactate and pyruvate production reflecting an inhibition of glycolysis irrespective of whether glycogen or added glucose was the substrate (Thurman & Scholz, 1977). Acetaldehyde metabolism during ethanol oxidation has been studied in perfused rat livers and observed ethanol metabolism was regulated by both the ethanol and acetaldehyde oxidation rates (Eriksson, 1977).

**Ethanol mediated electrophysiological changes**

Ethanol ingestion has an effect on the CNS. The electroencephalogram (EEG) reading is a measure of spontaneous electrical activity in the brain (Tabakoff & Hoffman, 1988; Devor & Cloninger, 1989). Ethanol use impairs the
performance of a variety of frontal lobe-mediated tasks, like those that require planning, decision making, and impulse control (Weissenborn & Duka 2003; Burian et al., 2003), but the underlying mechanisms are not known. Reports suggest that baseline blood flow to the frontal lobes increases during acute ethanol intoxication (Volkow et al., 1988; Tiihonen et al. 1994), metabolism in the frontal lobes decreases (Wang et al., 2000) and ethanol reduces the amount of activity that occurs when the frontal lobes are exposed to pulses from a strong magnetic field (Kahkonen et al., 2003). The evidence suggests that acute intoxication alters the normal functioning of the frontal lobe. EEG patterns have been shown to be different in ethanol addicts and controls. Monozygotic twins have been shown to have almost identical EEG responses to ethanol (Tabakoff & Hoffman, 1988). Subjects at high risk for ethanol addiction can be differentiated from controls on the basis of their EEG alpha activity (Pollock et al., 1983). Ethanol addicted subjects had greater increases of slow alpha activity and greater decreases of fast alpha activity after ethanol intake than controls. The high risk subjects also showed greater decreases in mean alpha frequency after ethanol intake. Neurophysiological measures, such as decreased P300 amplitude and altered EEG alpha activity, have been associated with increased ethanol addiction risk. The differences observed suggest that increased cortical P1 amplitude and altered cortical EEG activity in the 8-50 Hz frequency range may be neurophysiological risk factors associated with high ethanol consumption in mice (Slawecki, 2003). Kahkonen et al., (2003) reported that ethanol-induced differences were most pronounced at anterior electrodes.

In the present study control and ethanol treated rats were used to study the functional correlation of dopamine and serotonin through DA D2 & 5-HT2A receptor subtypes on ALDH activity. Real-Time PCR studies were carried out to
confirm the DA D₂ & 5-HT₂A receptor binding parameters. Perfusion studies were done to analyse the effect of dopamine, serotonin and glucose on ALDH activity. Also, the brain activity in ethanol treated rats was studied using electroencephalogram to assess the functional difference in this animal model.