Effective, well-researched treatments exist for most mental disorders, yet the majority of people who have severe mental illness are not treated. Mental illness ranks first in terms of causing disability in the United States, Canada, and Western Europe, when compared with all other diseases (such as cancer and heart diseases), according to a study by the World Health Organization in 2001. This groundbreaking study found that mental accounts for 25% of all disability across major industrialized countries. Throughout the world, scientist goal is to improve understanding, diagnosis, and treatment of mental illness. Medication and its harmful effects while treating mental disorder is an important part in mental ailment research. Particularly, use of drugs to treat mental disorder in pregnant women and ensuing teratogenic effect observed in offspring is a key research of interest (Cohen 1964).

An increasing number of studies draw attention to the effect of drugs taken by pregnant women on fetal development, especially the centrally acting drugs, which are the most commonly prescribed drugs for the management of psychiatric and psychosomatic complications (Ugaz et al., 1999; Khedun et al. 2000; Lorenti et al., 2003; Nicosia et al., 2003). These drugs are used during pregnancy for preeclampsia,
eclampsia, nausea, vomiting, mental tension, dizziness, anxiety and depression and hence inadvertent fetal exposure to these drugs is substantial.

Prenatal exposure to these drugs may manifest behavioural disorders in the offspring’s (Middaugh et al., 1975; Jaiswal and Bhattacharya, 1996; Singh et al., 1997; Fachinell et al., 2003).

In recent years, increasing attention has been paid to the effect of drugs on fetal development. Research in teratological effects of commonly prescribed drugs now, includes in addition to major birth defects, anatomical malformations, slight structural defects and functional impairment present at birth and in early infancy (Walz and Davis, 1979; Leonard, 1982; Khedun et al., 2000).

**ACTION OF BENZODIAZEPINES ON THE CNS**

The exact mechanism of action of benzodiazepines remains speculative. They probably facilitate the inhibitory presynaptic and postsynaptic action of GABA. The regional distribution of benzo receptors parallels that of GABA receptors in the brain. The antispasticity effect appear to involve the GABA receptors in the brain stem and the spinal cord, whereas the sedative and anticonvulsant activities are localized in the development of limbic system (Armitage, 1952; Cohen, 1964; Ananth, 1976; Holt et al., 1999; Tauber et al., 2003). Anxiolytic drugs have pharmacological actions similar to those of the older sedatives. The CNS depressant effect is dose dependent. In smaller doses, they relieve anxiety, but in large doses they induce sleep and can therefore be grouped together with sedative hypnotics. Because of this depressant action on the motor cortex, many of them also act as anticonvulsant.
Benzodiazepine derivatives are currently recommended for the treatment of anxiety. They include chloridiazepoxide, diazepam, oxazepam, lorazepam, prazepam, alprazolam and holazepam. Although these drugs share other therapeutic indications notably sedation and induction of sleep, there remains the possibility of later behavioural effects due to delayed development of central nervous system.

DZP is a benzodiazepine with central nervous system depressant properties and a somewhat flatter dose-response slope than the sedative-hypnotic drugs. In laboratory animals, it produces, in varying doses, taming, disinhibitory, sedative, anticonvulsant, muscle relaxant, ataxic and hypnotic effects.

DZP is relatively devoid of autonomic effects and does not significantly reduce locomotor activity at low doses, or depress amphetamine-induced excitation. In high doses, it activates the drug metabolizing enzymes in the liver. DZP also possesses dependence liability and may produce withdrawal symptoms, but has a wide margin of safety against poisoning. Metabolism studies in animals and man have indicated that oral DZP is rapidly absorbed from the gastrointestinal tract. Peak blood levels are reached within 1-2 hours after administration. The acute half-life is 6-8 hours with a slower decline thereafter, possibly due to tissue storage.

**EFFECTS OF DIAZEPAM IN HUMAN DEVELOPING CNS**

Chronic treatment of DZP during very early stages of life when nervous system is in a state of rapid development may result in long lasting structural and functional modification of brain (Nicosia *et al.*, 2003). In human, DZP produces sedation, calming effect and reduces aggressiveness. Exposure to the tranquilizer DZP
during gestation was reported to result in the so-called floppy infant syndrome characterized by hypotonia, hypothermia, respiratory complications, hyperbilirubinemia and poor sucking responses. (Armitage 1952; Flowers et al., 1969; Gauron and Rowley, 1969; Ananth 1972; Joyce and Kenyon, 1972; Rowlatt, 1978).

Human neonates whose mothers were treated chronically with DZP have exhibited symptoms of tremors, vomiting and irritability (Rementeria and Bhatt, 1977; Kolata, 1978). However, most of these conditions are short lived and infants generally recover sufficiently to be released from hospital with no attempt to monitor later behavioural sequence of the drug effect.

**ANIMAL MODEL STUDIES**

Animal model studies had shown that prenatal exposure to various neuroleptic and anxiolytic agents result in significant alterations of development and behavioural defects at some time or later. Behavioural alterations in offspring’s that were exposed prenatally to drugs (Kolata, 1978; Kellog et al., 1980; Ryan and Pappas, 1986; Khedun et al., 2000; Lorenti et al., 2003) and environmental chemicals were with increasing frequency of malformations, and functional disturbances.

In rats, rapid brain receptor development takes place in the third week of gestation and during first three weeks after birth (Braestrup et al., 1979). Paterson (1966) revealed degenerative changes in neuronal cells with perivascular cuffing characteristic of inflammation, infections, allergies and autoimmune diseases. Jacobson (1972) observed extensive gliosis (neural cell death) in the rat brain exposed to DZP and correlated the behavioural alteration in the fetuses. Similarly, Breen and
Stenchever (1970) observed degenerative changes in the neurons due to DZP treatment during pregnancy. Cavanagh and Condo (1964) had observed greater concentration of DZP in fetal plasma rather than in maternal plasma, because, DZP, the lipophilic drug with a small molecular weight, which crosses the placenta rapidly and the capacity of the fetus to dispose the drug is very small (Marcucci et al., 1973).

Winick (1974) observed rapid proliferation of brain cells differentiation and growth in neuronal tissue from about the 2\textsuperscript{nd} week of gestation until the third postnatal week. Ljubimov et al., (1974) observed prenatal alterations in maturation of spontaneous motor activity, delayed sexual maturation, impaired acquisition of conditioned avoidance responses in rats due to exposure of DZP during pregnancy.

Decreased postnatal survival and reduced offspring body weight due to oral administration of DZP in mouse were reported in certain studies (Guerriero and Fox, 1977; Gai and Grimm, 1982; Lauer et al., 1987). Conversely, Braestrup et al., (1979) have reported no changes in binding site of the whole brain, cerebral cortex, cerebellum or corpus striatum in the offspring’s of pregnant rats treated with DZP. Similar results were noted by Kellog et al., (1980) with DZP administration during last week of gestation. Barlow et al., (1979) observed auditory development disturbances in neonates treated with DZP during pregnancy.

Treatment of benzodiazepines during third week of gestation in rats showed interference with development of behavioural and receptor densities in the offspring’s (Gallager and Mallorga, 1980). Watanabe et al., (1983) have reported that pre and postnatal DZP exposure caused reduction in number of opioid receptors in the cortex.
and striatum of 14 days old rats. However, Kellog et al., (1983) had reported that DZP exposure is limited to specific and persistent suppression of nor-androgenic innervations of the hypothalamus. Further study revealed that, two months old offspring’s of DZP exposed rats exhibited substantial deficiencies in hypothalamic nor-epinephrine concentration (Simmons et al., 1984).

Similarly, reduced mid-brain norepinephrine content due to DZP treatment was reported by several workers (Detering et al., 1981; Middaugh et al., 1981; Frieder et al., 1984; Hill and Engblom, 1984). This showed prolonged prenatal exposure of DZP resulted in characteristic and fairly extensive pathological changes in the brains of mature rats.

Takeshi Shibuya et al., (1986) have observed early gestational treatment (1-12 days) of DZP might directly decelerate the development of opioid receptors in brain and then induce the hypersensitivity for pain, and further, it affects the variational development of startle responsiveness.

In rats, rapid proliferation of brain cells, differentiation and growth in neural tissue take place from about the second week of gestation until their postnatal week (Winick, 1974). Exposure of pregnant rats to DZP during the final week of gestation interfered with the development of arousal processes studied later in the pups (Kellog et al., 1980).

Exposure to DZP of rat dams throughout most of the gestation period (16 days) resulted in the pups showing reduced exploratory behaviour in an open field and a dose dependent learning deficit that manifested itself in a complex choice discrimination task, but not in simple learning at two months of age (Gai and Grimm, 1982).
Djeridane and Touitou (2003) looked at the effects of benzodiazepines on pineal gland melatonin secretion both in vitro and in vivo in rats. In vitro study showed no change whereas, in vivo study showed a single acute subcutaneous administration of DZP significantly affected pineal melatonin synthesis and plasma melatonin levels.

DZP was widely used in the treatment of anxiety and ethanol withdrawal. It has been suggested that this class of compounds may increase the reinforcing value of ethanol; yet, the literature is scarce. At non-sedating doses, DZP did not affect operant ethanol self-administration. At the higher doses, the drug suppresses ethanol self-administration in rat, but also induced significant suppression of locomotion, indicative of sedation (Rimondini et al., 2002).

OTHER SIDE EFFECTS IN MAN AND ANIMALS

**Congenital deformities**

Various teratogenic effects of DZP were discussed in literature via experimental and clinical studies, such as, increased risk of cleft palate in the offspring of man (Arskog, 1975; Safra and Oakley, 1975). Reports on long-term DZP therapy during pregnancy caused pronounced muscular hypotonia in the offspring called *floppy infant syndrome* (Gillberg and Speight, 1977). In another study, the acute metabolites readily cross the placenta (Idanpaan – Heikkila et al., 1971).

**Nutritional factor**

Healthiness of the individual determines the extent of the harmful side effects of the DZP. A study suggested that in the adults, the effect of DZP was more evident in the malnourished, when compared to the well-nourished one; malnutrition seems to
alter the brain responsivity to some cortical spreading depression-facilitatory or inhibitory agents in rats (Guedes et al., 1996).

### Carcinogenic effects

Carcinogenic effects of Benzodiazepine group are the important issue in detrimental effects of these drugs; certain studies do appear on the cancer inducing nature of DZP in the experimental animals. Horrobin and Trosko (1981) reported that DZP has the characteristics of a tumour promoter in a number of \textit{in vitro} systems and DZP accelerated the tumour growth in two different experimental animals. In their study, they also suggest that, the use of tranquilizer was found to be greater in women with metastatic breast cancer at the time of diagnosis than in those without metastases. They stress the need for further evaluation of the possible effects of DZP and related drugs on cancer in humans and animals.

While describing the hepatotoxic effect of DZP, Diwan et al., (1986) reported the development of hepatocellular hyperplastic foci and hepatocellular neoplasm (adenomas and carcinomas). They administered DZP and oxazepam to mice and found out that damage produced by DZP was more than oxazepam. Further, they reported that both DZP and oxazepam induced hepatomegaly, and they stress the need to study the effects of DZP on tumor development in different mammalian species.

The occurrence of neoplastic and non-neoplastic lesions may surface in long-term or later period; this was confirmed by the study conducted by Livezey et al., (1986) who demonstrated the neoplasm in rats aged up to 20 months. Although there
were no early postnatal effects of DZP, neoplasms developed in the DZP exposed rats resulted in the mammary fibroadenoma and uterine sarcoma.

**Genotoxicology**

Few studies were made on the genotoxic effects of DZP. Giri and Banerjee (1996) reviewed, the mutagenic and genotoxic effects of DZP and about the sensitivity to DZP in inbred mice and reported the possibility of genotoxic effects.

Mutagenic activity of DZP evaluated by *in vivo* cytogenetic tests done in mice indicate cytotoxicity. The results showed a significant increase in the frequency of micronucleated polychromatic erythrocytes at all doses. The frequency of sister chromatid exchange was significantly higher and concluded that DZP showed mutagenic and genotoxic effects on bone marrow cells of mice (Leal Garza *et al.*, 1998). Human health risk involved in the use of this drug also reported that the use of DZP reduces lymphocytes mitotic activity, causes numeric chromosomal aberrations (mostly hypodiploidy), and shows cytotoxic reactions in the culture of lymphocytes of human periphery blood (Ibrulj and Nefic, 1999).

A study of the induction of aneuploidy and chromosomal aberrations was conducted after DZP treatment using cultured hamster cells to assess the ability of four benzodiazepines, namely DZP, medazepam, midazolam and bromazepam, to induce numerical and structural chromosomal aberrations. It was observed that diazepam, medazepam and midazolam treatment produced dose-dependent reductions in the number of diploid cells, with medazepam and midazolam inducing significant levels of hyperdiploidy and DZP inducing low levels of
hypodiploidy (at toxic doses). In contrast, bromazepam treated cultures showed no significant changes in the level of aneuploidy even when exposed to toxic concentrations. This study suggests that, all four sedatives were seen to induce low levels of chromosomal aberrations, with bromazepam showing the most potent effect. These observations indicate that these structurally related benzodiazepines could be regarded as potentially genotoxic (Lafi and Parry, 1988).

Aneuploidy induction in male germ cells in men after chronic exposure to DZP was conducted. The study indicates that DZP acts as an aneugen during meiosis in male spermatogenesis, both in mice and humans. The quantitative comparison indicates that humans may be at least 10 times more sensitive than mice for aneuploidy induction by DZP during meiosis (Baumgartner et al., 2001).

**Effects on heart**

DZP has a direct myocardial depressant effect at the cellular level, which is mainly mediated by an inhibition of the sarcolemmal L-type Ca2+ channel (Juan-Fita et al., 2003). The actions of DZP inhibit the effects of noradrenaline in rat myocardium (Sugimoto et al., 1978; Nakae et al., 1997) demonstrated the direct effects of DZP on myocardial depression in cultured rat ventricular myocytes.

Similarly, in a vitro study by Kanaya et al., (2002) investigated the direct effects of DZP on cardiac excitation-contraction coupling in adult rat ventricular myocytes. A larger concentration of DZP (>300 micro M) nearly abolished the intracellular Ca(2+) and cell shortening or contraction. Though, the benzodiazepines have no direct influence on excitation-contraction of myocytes, at very large doses
this seems to produce alterations. These results indicate that the DZP differentially alter the cardiac excitation-contraction coupling at the cellular level.

**Genetic determinants of sensitivity to diazepam**

In an experimental study, conducted by Crabbe et al., (1998) on mice, who had tested for the sensitivity to effects of acute DZP exposure and reported that the sensitivity to the drug differs in different strains. These results suggest that there are multiple genetic determinants of behavioural sensitivity to DZP effects.

Another study done by Gallaher et al., (1987) on the DZP sensitivity and resistance showed that, the significant amount of difference observed in the duration of DZP induced neurological deficit between inbred mice, which were experimentally altered genes.

**Effects of Diazepam on immunodeficiency**

Literature indicates that the prenatal DZP treatment impairs immune responses in mammals. Prenatal period, is characterized by intensive histogenesis and cytodifferentiation of the already shaped organs. It is a highly vulnerable phase not only for fetal or neonatal brain, but also to immune system-organs (Livezey et al., 1986; Navarro et al., 1990; Teshima et al., 1990; Schlumpf et al., 1992; Ferrarese et al., 1993; Morgulis and Palermo-Neto, 2002).

Though certain pathologies are not evident at birth, but forms the basis for various functional defects of neuro-psycho-immunocompetence, which become apparent gradually during further maturation or even in adulthood. Clinical recognition of such functional teratogenic action of drugs is hampered by the long
time interval (up to decades) between the drug administration and its consequences, making the identification of causal relations very difficult (Dostal *et al.*, 1995; Galdiero *et al.*, 1995).

**MORPHOMETRY AND STEREOLOGY IN BIOLOGICAL RESEARCH**

Morphometry and stereology are terms used to denote quantitative analysis in scientific research. However, morphometry may regard as measurement related with morphology (like size of a cell or nucleus, diameter of the ducts etc.,) or gross measurement such as, weight, height, length, and so on. Where as, stereology is Mathematical science and which developed into a dynamic branch of quantitative research with lots of new innovation in the form of formulas and derivatives. Though, the terms morphometry and stereology were synonyms, stereology is generally defined as the procedure of deriving 3-dimensional information from measurements on 2-dimensional images. Morphometry can be defined as the quantification of structural features when the 3-dimensional structure is understood.

In the scientific method, a researcher will pose a hypothesis and then collect data to test the hypothesis. Medical researchers usually collect information about the number, length, surface area, and volume of specific aspects of their specimens for comparison. By examining and comparing these quantities, researchers can prove or disprove a hypothesis. However, an experiment can only be considered valid if the data have been collected in a reliable and unbiased manner.

In the investigation of structural and cellular differences between groups*, were often have to tell if one group is different than another by more quantitative methods than just "eyeballing" it. The subtle alterations between groups are often only
evident under analysis by the most stringent of analytical tools. Stereology makes quantitative analysis easy - whether looking at changes in cellular composition, or anything that can be viewed under the light microscope. This tool can help to quantify the structural and cellular composition of various groups. Conventional stereological principles and accepted morphometric procedures are outlined by Elias and Henning (1968); Elias and Hyde (1980); Cruz-Orive and Weibel (1990), it has come through a long way to the present stage. However, the use of quantitative technique in teratogenic effects of DZP were very much limited (Chakraborty et al., 1988; Chakraborty et al., 1992).

Consequently, the experimental research is necessary under the precondition of adequate animal models with sufficient validity for the extrapolation on human level. It is mandatory to employ such approaches, using drug application in neonatal rats with follow-up studies like cytoarchitecture of brain, behaviour, immune reactivity and brain biochemical analysis. The functional teratogenic risks in drug used in the treatment of risk pregnancies need thorough investigation.