DISCUSSION

SUITABILITY OF THE ANIMAL USED

Wistar Albino rat (*Rattus norvegicus*) was used in this study. The great adaptability and handiness of these species have made it suitable model for variety of research. This species is used for research in many disciplines such as, neurophysiology, endocrinology, biochemistry, pharmacology, toxicology, experimental physiology and oncology. Availability in adequate numbers provides the necessary quantitative assessment to make the data or result acceptable. Their broad similarity to man physiology is a further advantage. Hence with these advantages, Wistar albino rat was preferred as the experimental model for this study.

MORPHOLOGICAL STUDY

Pups exposed to DZP during full gestation or more than one trimester (group IV and V) exhibited a decrease in total body weight and brain weight. However, weight was not altered in group *I, *II and *III, indicating a dose dependent decrease in both body weight and brain weight. These results concur with the other studies in term of decrease in weights (Guerriero and Fox, 1977; Gai and Grimm, 1982; Lauer *et al.*, 1987). Conversely, other study has reported increase in body weight following the low dose of DZP (Stenchever and Parks, 1975). In the present study increase in body weight was not observed in any of the groups.
POSTNATAL ASSESSMENT

Physical developmental observation

The offspring’s were assessed on a number of developmental parameters. No differences were manifested for incisor eruption, surface righting, forward locomotion and vaginal opening or descending of testis after DZP treatment in any of the groups, confirms that DZP is not having any effects on these developmental processes. Whereas, in the case of ear opening, eye opening, retardation seen in the group *IV and *V indicating that delay in the developmental process due to DZP.

Behavioural assessments

Traction, Rotarod, midair-right reflex and cliff avoidance performance were affected in the prenatal DZP animals in group IV and V. DZP treatment during the very early stages of life, when the nervous system is in a state of rapid development, may result in structural and functional modifications of the brain. The results of the present study indicate that DZP treatment of pregnant rats induces significant changes in some aspects of development and behaviour of the offspring’s. Some of these changes are short lived and specific to age at testing (e.g., the slight retardation found around 21 days not seen after 30 days). The experimental evidence suggests that functional alterations were selective and defined by the neurochemical anatomy of the specific sites upon which the drugs act. Functional alterations may be expressed in childhood or later (Gai and Grimm, 1982) and major problem consequent to prenatal drug exposure may be a difficulty for the organism to meet specific challenges (Lall and Sahoo, 1990).
HISTOLOGICAL STUDY

The histological evidence suggest that early developmental exposure to centrally acting drugs can induce persisting neural and functional alterations in the offspring’s, which were reflected heavily in group *IV and group *V animals. Limiting the prenatal exposure to a single trimester reduced the severity of the lesions, but did not prevent their occurrences.

Observed lesions such as gliosis and perivascular cuffing provide some clues to the underlying mechanism in functional deficits. Perivascular cuffing is an inflammatory response and in general, it appears in infections and allergic reactions or in autoimmune diseases.

Although the mechanism responsible for the lesions were not known, many possible reasons were attributed to the presence of perivascular cuffing or infiltration in brain slices of rats. Extensive gliosis found in brain may be due to neuronal death (Jacobson, 1972; Frieder et al., 1984). DZP has been shown to cause some degenerative changes in cells (Breen and Stenchever, 1970; Frieder et al., 1984). Particles from such degenerative cells may induce inflammatory reaction intended to digest or remove the cellular debris.

Another possibility suggested by Frieder et al., (1984) was, induction of autoimmune disease. In normal condition self-antigen were recognized by the host during intra uterine life. In the case of DZP exposed animals, immuno-tolerance or response to self antigen may be suppressed or immuno-tolerance may be preponed, as the immuno-suppression after DZP was documented in rats (Livezey et al., 1986; Morgulis and Palermo-Neto, 2002).

Immunological barrier in the brain is highly effective. Not only it will protect the brain tissue from pathogens but also prevent leakage of brain tissue immunogen to
immunologically responsive centers and *vice versa*, a process that does occur when the blood-brain barrier is destroyed (Paterson, 1958; Kormano, 1967). Usually, barrier is weakened at the site of inflammatory autoimmune lesions (Barlow, 1956; Johnson, 1970) and barriers impair development of these lesions unless they are damaged or weakened locally (Levine and Wenk, 1967; Johnson, 1970). In the present study, the presence of DZP prenatally impairs blood-brain barrier leading to inflammatory reaction.

Other possibility was, normally fetuses are protected by immune system of the mother. Mothers treated with DZP would be short of immune protection by the presence of drug, leading to lack of protection to the pups. Entry of pathogens to the fetus was facilitated by the altered permeability of placental membrane due to DZP effect (Breen and Strenchever, 1970; Frieder, *et al.*, 1984).

**HISTOMORPHOMETRIC STUDY**

Histomorphometric study revealed a reduction in width of the cortex of experimental pups, which might be due to the increase in the packing density of the cortical neurons, as well as smaller size of the cortical neurons and less amount of intercellular substances. Pups of the control animals showed four distinct layers of cellular arrangement, whereas in the case of drug treated animals the number of distinct layers were reduced to three. These alterations in the cortex of drug treated animals may be due to the inactivation or faulty developmental process, induced by DZP. Both cortical width and neuronal packing density changes were statistically significant. The degree of alterations varied in different groups, depending on the dose and the period of administration.
OTHER SIDE EFFECTS

The possible effect of DZP on cancer development and growth are still unknown. Although in this study, brain showed extensive lesions, none of the drug treated animals showed signs of tumour like lesions in brain. However, DZP has the characteristics of a tumour promoter in a number of in vitro systems (Horrobin and Trosko, 1981). The effect is apparent at concentrations of DZP, which are clinically relevant, would accelerate tumour growth in two different experimental animal cancers (Giri and Banerjee, 1996). Further evaluation of the possible effects of DZP and related drugs on human and animal cancers is urgently required.

GENOTOXIC STUDY

Chromosomal Aberration

Result indicates that DZP treated animals showed chromosomal aberration when compared to the control animals. But the frequencies were highly varied between animals of different group. The observations clearly indicate DZP is capable of inducing numerical chromosome changes such as aneuploidy or polyploidy, and in certain chromosomes morphological alteration such as deletion or fragmentation were obvious.

Micronucleus test

The bone marrow cells were processed for the micronucleus tests. Micronuclei are fragments of chromosomes or whole chromosomes, which fail to get
incorporated into daughter nuclei during mitosis either due to spindle position or lack of centromere (Schmid, 1975). Usually, the Microneuclei expressed in cells, which have completed their first mitotic division in response to mitogen stimulation. The Microneuclei in cells were recorded by blocking the cytokinesis stage during their first mitotic division (Fenech and Morley, 1985). The micronuclei were more in cells obtained from drug treated animals in group *IV and group *V. These results clearly indicate that the effects of DZP on dividing cell could impede normal cell division under chronic treatment.

BIOCHEMICAL STUDY

Plasma sodium and potassium fell significantly in the group *IV and *V indicating a dose dependent alteration in the ionic balance. Similarly in the brain, the drop in the level of the sodium and potassium ions indirectly indicates alterations due to DZP administration. According to Nicosia et al., (2003), prenatal treatment with small doses of DZP may counteract the effect of physical stress in rats, normalizing the time, course of neonatal reflexes and the behavioural responses in adulthood. However, prenatal administration of DZP in greater doses may induce desensitisation of gamma-aminobutyric acid A (GABA(A)) receptors, which might be reflected in brain by means of alterations in sodium and potassium levels.

In this present study, certain lesions were found only in some animals of a particular group, while others in the group showed some resistance to the drug. This is in accordance with the studies of Crabbe et al., (1998) who worked on mice showed that the sensitivity to the drug differs in different animals. These results suggest that there are multiple genetic determinants of behavioral sensitivity to DZP
effects. i.e., genetically influenced sensitivity to DZP is not massive but is somewhat specific to the particular neuronal response.

Number of studies in adult animals suggest that chronic DZP treatment result in CNS suppression with impairment (Cassone and Molinengo, 1981; Grimm and Hershkowitz, 1981). However, retardation in the behavioural assessments seen in the present study cannot be explained as direct effect of the drug, rather, it is the modification in the brain during early development which was substantiated by the histological and histomorphometric findings.

Demonstrating the existence of these lesions may have important implication concerning the therapeutic use of DZP during pregnancy and early development. This study also necessitates a careful evaluation of this literature with respect to its relevancy to humans. Such information should guide future clinical investigations.