

## ABSTRACT

## MOLECULAR STUDY OF OCCURRENCE CLEFT PALATE DUE TO DIAZEPAM INTAKE IN MICE PRENATAL THROUGH EXPRESSION OF GABA, CASPASE-9, BAX AND APOPTOSIS

This study aims to explain the occurrence of cleft palate in the embryos of mice due to intake of diazepam in the period of organogenesis by analyzing changes in GABA levels and expression, protein expression of caspase-9, Bax and Apoptosis.

About 27 mice of known age and weight were used. After pregnancy, they were divided in three groups: the first groups were control group which were injected daily with distilled water. The second groups that were injected daily Diazepam 8 mg/kg/day and the third injected daily with Diazepam 16 mg/kg/day. All the above groups were administered intraperitoneally injection during the organogenesis phase (9<sup>th</sup>-15<sup>th</sup> days)

On the 15<sup>th</sup> of gestation period all the pregnant mice were sacrificed and the embryos were studied macroscopically for anomalies and then the tissue were fixed and processed, stained and examined microscopically. Anomalies of cleft palate were evaluated. Apoptotic cells in tissue section were identified by TUNEL Assay and for expression of GABA, caspase-9 and Bax were identified by immunohistochemistry. Examination GABA levels by ELISA and protein expression of caspase-9 and Bax were observed using SDS-PAGE and Western Blotting.

The results of the detailed microscopic analyses on the embryos of the first and second test groups show that the cleft palate appeared to be a complete failure of the second palate formation. There are significant differences ( $p < 0.05$ ) on all variables observed between control and treatment groups except the expression of GABA test group I and controls (0.084). Increased GABA levels significantly in both test groups. Protein caspase-9 and Bax was detected specifically in the range of molecular weight of 49 kDa and 20 kDa.

Conclusion: The exposure to diazepam during the period of organogenesis causes *cleft palate*, with inhibition of shelves elevation caused an increase GABA and apoptosis activity through the intrinsic apoptosis path.

Keywords: diazepam, cleft palate, apoptosis, GABA, caspase-9, Bax, information