

# Effects of Maneb (Manganese-BisDithiocarbamate) in cap stage of tooth development in rats

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**Abstract:** Objectives: Maneb contains manganese (Mn), is a fungicide, a nutrient that becomes toxic to humans at high exposures. It has been assumed that mane's potential for carcinogenicity is due to the formation of the metabolite. The purpose of this study is to investigate the histopathologic and molecular changes in tooth structures in the infant rats whose mothers have been treated with mane during pregnancy.

**Study design:** In this study, pregnant female rats received 250 ppm propineb concentrations in 5 ml distilled water for 16 days of gestation, and then infant rats were obtained by cesarean section. In the histological analysis on the frontal sections, the use of mane was found effective on odontoblast cell hypertrophy and degeneration in the ameloblast cells. The expression of MMP-2 (Matrix-Metalloproteinase-2) and VEGF (Endothelial cell growth factor) in the connective tissue was evaluated by immunohistochemistry.

**Result and conclusion:** We conclude that, depending on its Mn effects, Odontoblast cells on the field during formation of dentin and enamel formation was observed when the cells to be effective ameloblasts. It is thought to be effective on the development of ectoderm cells.

**Key words:** Maneb, tooth development, cap stage, VEGF, MMP2



## Introduction

Tooth development is highly regulated through a series of reciprocal and sequential epithelial–mesenchymal interactions that lead to cuspal morphogenesis, cell differentiation, and the subsequent formation of the specialized matrices of enamel, dentin, cementum, and bone (1,2,3). The dental follicle surrounding the tooth germ contains mesenchymal cells that are able to differentiate into osteoblasts, cementoblasts, and fibroblasts (4). The human tooth as a bio-matrix of past exposures to metals was detailed in a recent review, which also discussed tooth components and tooth development process (5). Few studies have been carried out on the mechanisms of organometallic fungicide action or on the fate of fungicides in target organisms. However, many studies have been reported on the effects of heavy metals alone in a variety of organisms (6). Pregnancy and the first year of life are potentially

vulnerable periods of exposure because Mn crosses the placenta during pregnancy, and young children have increased absorption efficiency and reduced excretion via bile compared to adults(7).Manganese is used in agricultural pesticides, industrial, and fuel additives. It is originate in air, soil, water, and food(8).

Mn is important to human health, acts as a co-factor for enzymes, and is required for normal development. Additionally,Mn is interested in regulation of blood sugar and vitamins, and care of nerve and immune system.Manganese (Mn) is an essential nutrient, however, can be toxic to many organ systems and across different life stages at high level. Especially, it may be harmful to the neurological system (9).

Multiple studies have reported associations between exposure to Mn and neurodevelopmental problems in children. Higher in utero Mn levels measured in blood and teeth have been associated with attention problems (Ericson et al., 2007; Takser et al., 2003), behavioral disinhibition (Ericson et al., 2007), impaired non-verbal memory (Takser et al., 2003), and poor cognitive and language development (Lin et al., 2013) in toddlers and preschoolers, and with externalizing behavior and attention problems (Ericson et al., 2007) in school-aged children. Postnatal Mn exposure has been associated with poor language development in toddler boys (Rink et al., 2014), and behavioral problems in schoolaged boys and girls (Ericson et al., 2007).(10xxx)Manganase(Mn) is incorporated directly into developing dentin and current analytical techniques allow for detailed Mn measurements that can be related to specific time periods of neonatal development beginning in the second trimester of pregnancy for incisors (13–16 weeks gestation) and ending 10–11 months after birth for primary coronal dentin in molars(11).During the odontoblastic processes, MMP-2 may be concentrated in an area adjacent to the dentino-enamel junction and, in this way, may contribute to these processes (12).Vascular endothelial growth factor (VEGF) potentially regulates the formation of new blood vessels through VEGF receptors and has been reported to be a key factor in periodontal angiogenesis during tooth movement(13).The purpose of this study is to investigate the histopathologic and molecular changes taking place in the early stages of tooth eruption in the infant rats whose mothers have been treated with maneb during pregnancy.

### **Material and Method**

Twenty pregnant female Wistar albino rats of initial body weight of 200–215 grams were divided into two groups (five animals for each exposure group and five females used as control). Females were mated with non-exposed males; the criterion used to identify mated females was the presence of spermatozoa in the vagina (day 0 of gestation).The animals were group-housed (10 per cage) under standard conditions ( $21 \pm 2$  °C) in the Animal Health and

Research Center of Dicle University (DUSAM). The animals had free access to standard laboratory rat pellet and water. Two groups of pregnant females (day 0 of gestation) received separately 250 ppm maneb (tech. 87.8% purity, obtained from Bayer and Hoechst-Schering, respectively) concentrations in 5 ml distilled water 5 days per week until the end of gestation. The controls received only distilled water. Doses administered orally by gastric tube (helping toxicant injections orally) are calculated on the basis of initial body weight of each animal (ppm=mg toxicant per 1 kg body weight). Acute oral LD50 values for male rats have been found to be 6750 mg/kg for maneb (14). Pregnant females were treated with the maneb for 21 days and litters were sacrificed at first day of birth. Maxillary and mandibular regions of litter rat were dissected under ketamine hydrochloride anesthesia. The samples were placed in 10% formaldehyde and dehydrated in 70–100% ethanol series. They were then placed in paraffin baths at 58°C for paraffin inclusion. Sections of 4–6  $\mu$ m were prepared from paraffin blocks using a rotary microtome. These sections were then stained with trichrommasson

### **Immunohistochemical analysis of MMP2 and VEGF**

Antigen retrieval process was performed twice in citrate buffer solution (pH: 6.0); the first for 7 minutes, and the latter was boiled in microwave oven at 700 W for 5 minutes. They were allowed to cool to room temperature for 30 minutes and washed twice in distilled water for 5 minutes. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 20 minutes. Ultra V block (Cat.No:85- 9043, Invitrogen, Carlsbad, CA,USA) was applied for 10 minutes prior to the application of primary antibodies and polyclonal antibody against MMP-2 (1/200) (Neomarkers Co., Inc., USA) (vWF antibody, rabbit-anti-vWF, 1/ 800, ab6994, Abcam) overnight. Secondary antibody was applied for 20 minutes. The slides were then exposed to streptavidin–peroxidase for 20 minutes. As a chromogen, diaminobenzidine (DAB: Invitrogen, Carlsbad, CA, USA) was used. The control slides were prepared as mentioned above with the omission of primary antibodies. After counterstaining with hematoxylin and washing in tap water for 8 minutes and in distilled water for 10 minutes, the slides were mounted with entellan.

### **Result and Discussion**

The control group and the group treated maneb were evaluated by histological sections and immunohistochemistry. At 16 day intrauterine life, morphogenesis proceeds as the epithelial cells proliferate and form the enamel organ surrounding the mesenchymal dental papilla and the tooth germ in the cap stage. The transition from bud to cap stage is characterized by the development of the cervical area. The dental epithelium appears, and the horseshoe-shaped dental lamina generates the future dental arches at the late cap stage. The

dental mesenchyme cells that are condensed around the bud formed the dental papilla between the dental follicles surrounding the epithelium. The external dental epithelium was formed by several layers of cubical cells and the internal dental epithelium by only one layer (Fig. 1a).development and degeneration of mesenchymal cells. In the group with treated manebdegeneration in ameloblast cells, odontoblasts cell hypertrophy also showed dilation of blood vessels in the dental papilla(Fig. 1b).. In the group treated maneb, an increase in blood vessels in VEGF expression has shown the dental papilla(Fig. 1c).Maneb is thought to induce the angiogenic activity of vascular development in the dental papilla.In the group with treated maneb , decreased MMP2 in the odontoblasts during the cap stage of tooth development was found effective on the formation of collagen in the basement membrane deposition of dentin (Fig. 1d).

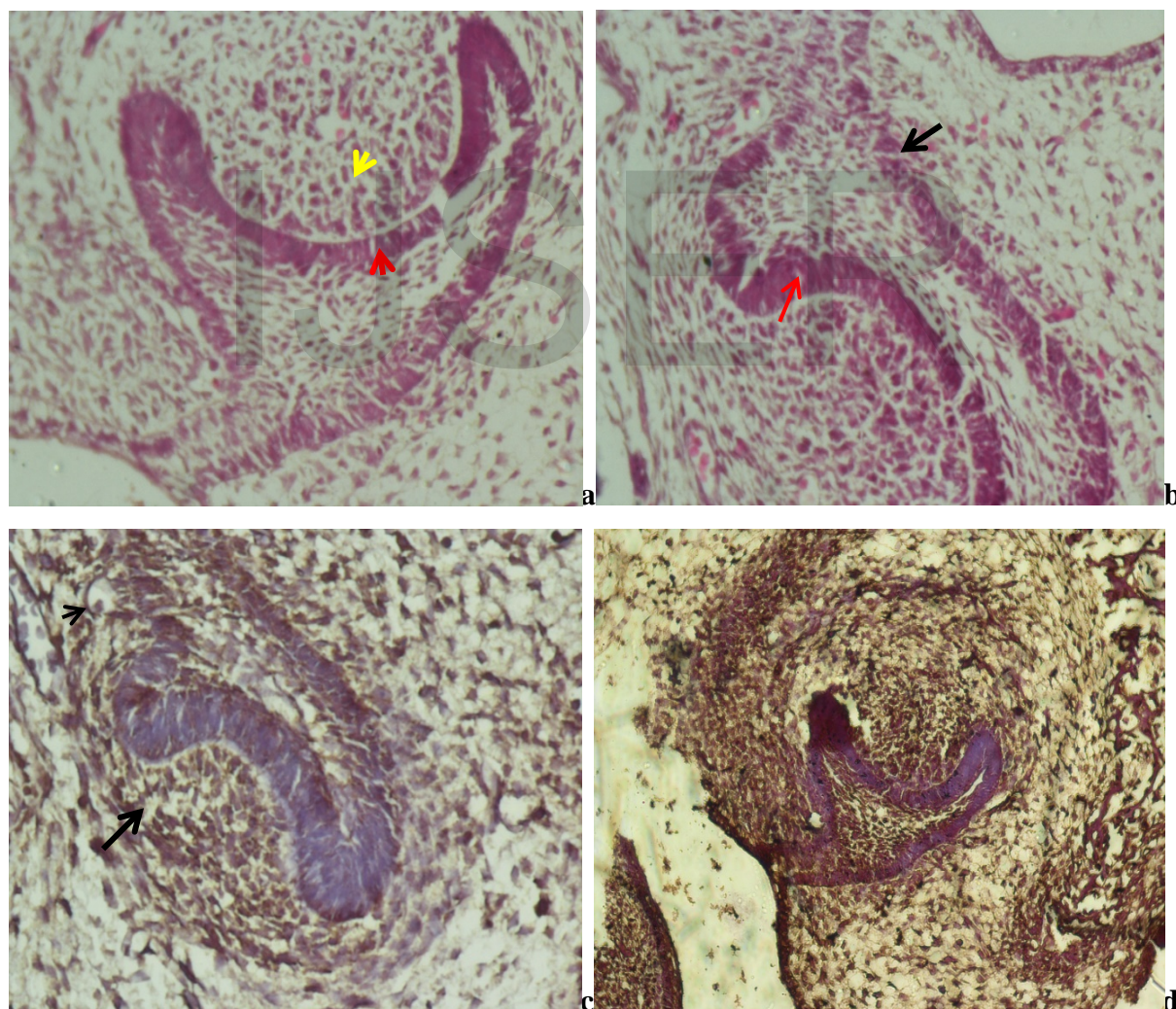


Figure 1 a- Control group:, Odontoblasts (red arrow), dental papilla mesenchymal cells (yellow arrow) in the normal developmental differentiation,H-E Bar 100μm,Figure 1b- The group with treated maneb, Degeneration in ameloblast cells(arrow),the slowdown in dental



capstage, Hypertrophia in odontoblast cells (red arrow), H-E Bar 100 $\mu$ m, Figure 1c- The group with treated maneb (VEGF expression) A increase of VEGF expression in blood vessels and mesenchymal cells around of dental papilla (arrow), VEGF immunostaining Bar 100 $\mu$ m, Figure 1d- The group with treated maneb (MMP2 expression) Negative MMP2 expression in odontoblast cells

The studies of medicine and dentistry are often used in studies of different animal groups before human (15). Particularly, rats which the experimental animal are used in most of pharmacological studies. Pharmaceutical products that preferred as systemically are given method of orally like in our study (16).

Maneb is a toxic agent to humans. It enters the body mainly through the respiratory tract (aerosol, dust) skin and mucous membranes. Local allergic reactions (dermatitis, conjunctivitis, rhinitis and bronchitis) (17), CNS effects (18,19) and accumulation in the liver (20).

Previous studies have used measurements in tooth enamel to estimate Mn exposure; however measurements in enamel cannot be readily linked to developmental timing of exposure because, unlike dentin, initial deposits of enamel matrix are not completely mineralized immediately but rather more slowly and diffusely during maturation (21). VEGF expression in pulp fibroblasts and odontoblasts of human teeth is higher in immature than mature permanent teeth, suggesting a role of VEGF in tooth maturation (22).

The expression and activity of MMP-2 increased progressively with the development of the tooth germ, and were found mainly in the dental papilla. This gradual increase of MMP-2 correlates with a period of rapid morphological alterations in the dental papilla, which changes from a soft myxoid-type connective tissue found in neonates to a more fibrous stroma present in 15-day-old rats (23). Development of teeth is regulated by interactions between the epithelial and underlying mesenchymal tissues. Reponen et al. (24) demonstrated that MMP-2 is expressed in the mesenchyme of developing organs including early developmental stages of the tooth, and it is strongly upregulated in differentiated odontoblasts at the time of basement membrane degradation. Koparal et al, propineb application of enamel matrix layer is stated that the delay in development and degeneration of mesenchymal cells (25). We conclude that, depending on its Mn effects, Odontoblast cells on the field during formation of dentin and enamel formation was observed when the cells to be effective ameloblasts. It is thought to be effective on the development of ectoderm cells.

In a previous study has identified high level Mn in children's prenatal dentin whose mothers worked in agriculture during pregnancy and use of Mn containing agriculture pesticides near

of their home. (8). Additionally, in study has been found higher Mn levels in prenatal blood and children's urine. (8). We found that in the group with treated Mn contain agricultural pesticides (maneb) degeneration in ameloblast cells, odontoblasts cell hypertrophy also showed dilation of blood vessels in the dental papilla.

Mora et al. found that that Mn levels in dentine of primary teeth were negative associated with behavioral results, internalizing, externalizing, and hyperactivity problems. , in school-age boys and girls ( 10).

Finally, we argue that maneb risk of for human teeth health. In particular, people who are nearest to human habitation agriculture, and families of agricultural workers. Farmers should be educated use of both the maneb and other pesticides. They should be informed about damage to cause of environment of the pesticides. Moreover, people who are nearest to human habitation agriculture and families of agricultural workers should be informed about pesticides. We suggest that making more research on this topic be useful.

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