

# Effect of Mancozeb Inhalation on the Olfactory Mucosa in Rats

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## Abstract

**Background:** Mancozeb, (ethylene-bis-dithiocarbamate), is an important fungicide useful against a wide range of fungus affecting ornamental plants, crops, and fruits. We aimed to evaluate the changes in the olfactory mucosa due to mancozeb toxicity and, if cytokine is active, IL-6 immunoactivity. **Material and Method:** In experimental group, the mancozeb (500 mg/kg) was administered with inhalation to 10 male Wistar Albino rats for five days a week. The control group (n = 10) received distilled water with spray at the same time period. The experiment was terminated after three weeks. Samples were placed in 10% formaldehyde for fixation and placed in paraffin, sections of 5 µm were prepared from paraffin blocks and stained with Hematoxylin-Eosin. Interleukin-6 (IL-6) primary antibody were used for immunohistochemical analysis. **Result:** In the mancozeb group, olfactory epithelial cell degeneration and apoptosis, inflammation, dilatation and congestion in the vessels were observed. IL-6 expression was increased in vascular endothelium and inflammatory cells. **Conclusions:** Mancozeb was thought that the increase in IL-6 expression due to the increase in cell degeneration signal was thought to affect the development of cell apoptosis and angiogenesis, and that the use of mancozeb might adversely affect the olfactory mechanism.

## Keywords

Mancozeb, Olfactory Mucosa, IL-6, Rat

## 1. Introduction

The nasal cavity is the entrance part of the respiratory tract and is an important issue with a wide mucosal area and a dense vascular network that completely takes the inhalation in the air. The nasal airway is highly susceptible to infection

and inflammation. A damaged nasal mucous membrane causes discharge, congestion and swelling. The nasal mucosa can also be an important target for many toxic substances inhaled in air pollution. Organosulfur compounds, a group of ethylene-bis-dithiocarbamates (EBDCs), such as maneb, zineb, and mancozeb, are widely used in pre-harvest agricultural applications. Mancozeb is a widely used broad spectrum fungicide that controls many fungal diseases in a wide range of field crops, fruits, and etc. Mancozeb exposure is common among workers who produce the chemical and also among agricultural workers after inhalation of dust or fine spray, dermal contact, or accidental/incidental ingestion, as in eating or smoking before washing hands [1]. Manganese has been reported that the transport of manganese through olfaction causes neurological dysfunction—especially its effect on the sense of smell, decreased sense of smell (hyposmia) and other olfactory disorders [2]. Moberly *et al.* [3] showed that acute intranasal administration of manganese in mice caused a 90% reduction in odorant evoked neurotransmitter release. Interleukin (IL)-6 is one of the cytokines that can inhibit Th1 cell differentiation and contribute to the pre-inflammatory process by inducing Th2 cells, especially in allergic reactions [4].

We aimed to evaluate the changes in the olfactory mucosa due to mancozeb toxicity and, if cytokine is active, IL-6 immunoactivity.

## 2. Material and Method

**This study was approved by Dicle University Local Ethical committee with protocol number 2021/03. The animals were obtained from Dicle University Medical Sciences and Application Center.** Adult Wistar albino rats were used in this study. There were 10 rats in the control group and 10 rats in the experimental group. Mancozeb was achieved by squeezing of mancozeb spray inside it. Animals were kept in the glass vase for 1 hour. Mancozeb (500 mg/kg) was administered with inhalation to 10 male Wistar Albino rats for five days a week at non-exposure times, rats were kept in laboratory animal house, which was far from the place of exposure with no maneb detection. The control group (n = 10) received distilled water with spray. At the end of the study, the animals were sacrificed by decapitation under ketamine hydroxide anesthesia. The skins were removed as well as all the soft tissues surrounding the nasal cavity. Then, the bony framework of the nasal cavity including nasal septum were nibbled out by bone-nibbler. The nasal region were fixed with zinc-Formalin solution and decalcified with 5% EDTA (Ethylene-diamine tetra acetic acid) [5]. Tissues were passed through ascending alcohol series for about 12 hours. Tissues were washed with xylene 2 × 25 minutes and incubated within paraffin wax. 4 - 6 µm sections were cut with microtome. Sections were stained with routine Hematoxylin and Eosin [6].

### *Immunohistochemical technique*

Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized in xylene and

brought to distilled water through descending alcohol series. The antigen retrieval process was performed twice (for 7 min and 5 min, respectively) with EDTA buffer solution (pH 6.0) in a microwave oven at 700 W. The sections were allowed to cool at room temperature for 30 min and washed twice in distilled water for 5 min. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 20 min. Ultra V block (Cat. No. 85-9043, Invitrogen, Carlsbad, California, USA) was applied for 10 min prior to the application of primary antibody IL-6. Secondary antibody was applied for 20 min. Slides were then incubated to streptavidin-peroxidase for 20 min. as chromogen, diaminobenzidine was used. Control slides were treated with same procedure, but PBS was used instead of the primary antibodies. After counterstaining with Hematoxylin and washing in tap water for 8 min, sections were examined in light microscope [7].

### 3. Result

The histopathological results of the present study were evaluated under light microscope. There were not any histopathological changes in control group sections of olfactory mucosa

Transversal section of the olfactory mucosa: Although vacuolar structures were observed in some epithelial cells, it was observed that the nuclear structures were more regular and regular, especially the basal and support cells were prominent. It was observed that there were especially few leukocyte structures in the lamina propria, where the glands are predominant, but the vascular dilations did not progress excessively, and the core structure of the glands was generally flat and cubical, rich in chromatin (**Figure 1**). While no changes were observed in the parallel and sometimes vertical sections of some fibrous structures, no changes were observed in the bone structure. In the histological section of the concha nasalis superior, local degenerations (arrow) and shrinkage in the nuclei, occasional vacuolization in the epithelium and emptied structures in the cytoplasm (arrowhead) were observed in the basal cells, especially in the basal region, and in the supporting cells in the olfactory mucosa.

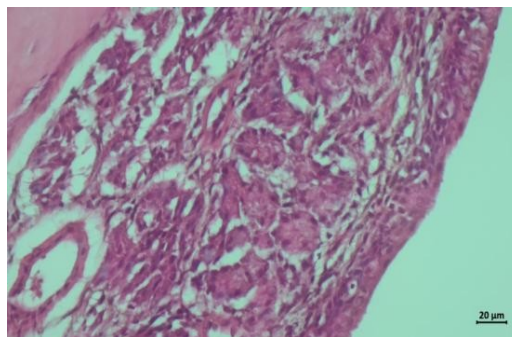
When we look at the lamina propria, excessive dilatation (star) in the blood vessels and thinning of the vessel wall, vacuolar structures are frequently seen in the ducts of the glands, degenerative changes in the seromucous glands (hollow stars), inflammation in the form of aggregates and hyalinized areas in the deep parts of the lamina propria towards the lower regions (blue stars). Leukocyte infiltrates (blue stars) scattered in solitary styles were seen on the upper sides. Nothing was seen in the bone trabeculae in the nasal region (**Figure 2**).

**Control group IL6 staining (Figure 3):** In the histological section of the olfactory mucosa, negative interleukin expression was observed especially in epithelial cells (arrow), while expression was positive (arrow) in cells with inflammatory properties at the bottom of some glands. In general terms, interleukin expression in the lamina propria region was evaluated as negative.

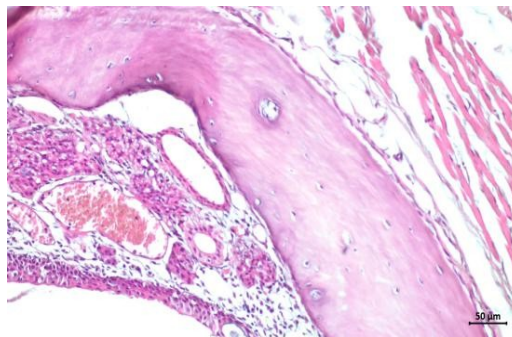
**Mancozeb Group IL6 staining (Figure 4):** It was observed that interleukin

expression started to increase especially in basal cells where olfactory epithelium is present, and inflammatory cells approached basal cells and the expression of interleukin was positive (arrow) in this section. Interleukin 6 expression was evaluated as positive (arrow) in cells where some glands are located in the lamina propria, Expression in vascular endothelial cells was positive in some places. In general, inflammatory cells in the form of small aggregates increased in the lamina propria region and an increase in positivity with interleukin 6 (asterisk) was observed.

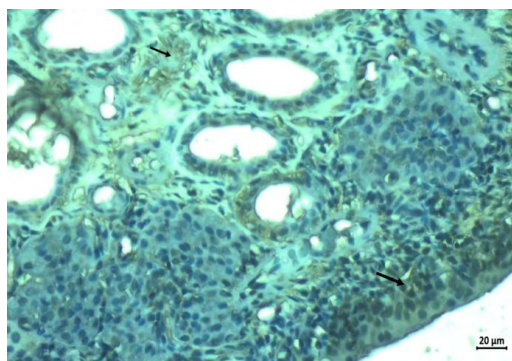
An immune score of IL6 expression was done by a modified version of Taş *et al.* study [8]. 5 samples for each group were analyzed and 5 fields in each sample were evaluated. For scoring, 0: negative, 1: weak positive, 2: positive, 3: strong positive.



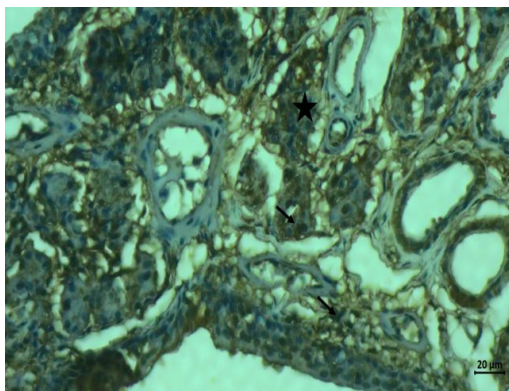
**Figure 1.** Control group.



**Figure 2.** Mancozeb group.



**Figure 3.** Control group.



**Figure 4.** Mancozeb group.

#### 4. Discussion

Damage to the respiratory mucosa occurs with the inhalation of toxic gases and various particles. The nasal mucosa is the first entry site for toxic substances. In the studies, the main histopathological changes in the respiratory mucosa due to the effect of toxic agents are edema, inflammation, fibrosis, mucosal ulceration, necrosis, hyperplasia, squamous metaplasia and neoplasia [9]. The reaction of certain natural substances (urea, glycine, oxalic acid, and imidazoline) with Mancozeb causes these molecules to affect blood-soluble enzymes and indicators of stress conditions, leading to a condition that causes several cell pathways to be altered [10]. Inflammatory parameters that change protein and fat metabolism such as urea, creatinine, hypoalbuminemia, electrolyte disorders and some liver enzymes (GOT, GPT and LDH) increase [11]. Nasal mucosa shows inflammatory response, fluid extravasation, mucus hypersecretion, edema and mucosal deterioration against inhalation of toxic substances. Cytokines come into play and determine the pathological process. Stem cells in the basal layer of the olfactory epithelium have the ability to regenerate the neuroepithelium even after significant damage. In a pilot study, intranasal manganese administration increased the Olfactory epithelial and olfactory ampule manganese concentrations; and intranasal manganese exposure has been reported to impair the performance of pre-trained rats in the non-moving olfactory discrimination (OD) task [12] In our study, a significant degeneration of epithelial cells in the olfactory mucosa and an increase in apoptotic cells as a result of disruption of the nuclear structure were observed in the histopathological examination due to the effect of Mancozeb (Figure 2). It was thought that this might be due to the manganese effect.

**Table 1.** Scoring of IL-6 expression in groups.

Parameter	Counted fields (each group)	Control group Median (Min-Max)	Mancozeb group Median (Min-Max)
IL-6 exp.	25	0.5 (0 - 1)	3 (2 - 3)

Exp: expression.

IL-6 is a proinflammatory cytokine and plays an important role in the local or systemic inflammatory process. Inflammation of the olfactory epithelium, submucosa, and nasal sinuses has been reported in rats given manganese. Inflammatory lesions seen in rats following manganese inhalation have been reported to occur primarily in areas of high airflow, but reversible upon cessation of exposure, resulting in mild irritation. In our study, the cytokine response to the increased inflammation with mancozeb inhalation was clearly demonstrated in the epithelial cells and connective tissue cells with the increase in IL-6 expression (Figure 4, Table 1).

## 5. Conclusion

In conclusion, mancozeb was thought that the increase in IL-6 expression due to the increase in cell degeneration signal was thought to affect the development of cell apoptosis and angiogenesis, and that the use of mancozeb might adversely affect the olfactory mechanism.

## Limitations

For the apoptosis evaluation, an TUNEL assay could be performed.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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