Histological study of the effect of eucalyptol oil vapours on the development of the palate and tooth germ (experimental study on rats)

Muhanad T. Jehad, B.D.S., M.Sc. (1)  
Athraa Y. Al-Hijazi, B.D.S., M.Sc., Ph.D. (2)

ABSTRACT
Background: Eucalyptol is a natural organic compound. The aim of this study was to evaluate the effect of the eucalyptol oil vapour on the palate and tooth germ development of rats embryos for the periods of (16th day, 18th day intrauterine life and one day old rats) histologically and histomorphometrically.

Materials and Methods: In this study thirty pregnant albino Wistar female rats (2-3 months of age, 200-250 gm of weight) were divided into two groups: Control group not subjected to the synthetic eucalyptol oil inhalation vapour and experimental group subjected to the synthetic eucalyptol oil inhalation vapour. The embryos at 16, 18 day of intrauterine life and one day old were histologically studied for the development of palate and molar tooth germ development.

Results: The results demonstrated a retardation of the palate and tooth germ development of 16th and 18th intrauterine life embryos of experimental groups in comparison to control. Immature enamel, wide predentin and interglobular dentin were detected in the tooth germ of embryo (one day old) from pregnant rats exposed to the eucalyptol oil vapour.

Conclusions: Eucalyptol oil vapour can affect on the palate (failure of fusion of palatine shelves) and tooth germ development (mineralization and maturation process of dentin and enamel respectively showing immature enamel and interglobular dentin with wide predentin). 

Key words: Eucalyptol oil, Wistar rats, palate, molar tooth germ, palatine shelves.

INTRODUCTION
Eucalyptol is widely distributed in plants. The main food sources are eucalyptus oil (up to 80% eucalyptol), the herbs and spices mugwort, sweet basil, rosemary, sage, cardamom and their essential oils. Eucalyptol is a monocyclic terpene with an ether bridge between carbon 1 and 8. Eucalyptol, 1, 8 cineole, is an essential oil, present in large amounts in a variety of plants which is frequently used in the manufacture of cosmetics to increase percutaneous penetration of drugs, as a nasal decongestant and anticoagulant, in aromatherapy, and in dentistry. Eucalyptol has been used to treat bronchitis, sinusitis, chronic rhinitis and for the treatment of asthma. These actions seem to be related to an anti-inflammatory action inhibiting the production of tumor necrosis factor alpha (4).

Gasric protection preventing ethanol-induced injury was reported in rats. This compound also enhances blood circulation, leading to skin hyperemia after local application.

Other reports showed that prolonged exposure to eucalyptol (inhalation); increases cerebral blood flow correlated with eucalyptol concentration in blood, suggesting a vasodilator action. Cardiovascular effects were also recently reported (2) showed that eucalyptol reduced heart rate by a parasympathetic-dependent action and induced hypotension by a direct vasodilation relaxation. Eucalyptol is metabolized to 2-exo-hydroxy-1,8 cineole by microsomes from human and rat liver, but it is not clear whether this substance can be metabolized by humans, in vivo. Eucalyptol diffuses faster by inhalation than by oral administration or through the skin. Its presence can be detected in blood 5 min after inhalation, with maximal concentration being reached within 18 min. Non-fatal symptoms were observed in children following nasal administration of eucalyptol. Effects included mucous membrane irritation, tachycardia, dyspnea, nausea, vomiting, muscle weakness, somnolence, and coma (3).

The oil is found in numerous over-the-counter cough and cold lozenges as well as in inhalation vapors or topical ointments.

The oil has antifungal and antibacterial activity against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Eucalyptus oil is generally nonirritating, nonsensitizing and
nonphototoxic to the skin. Essential oils are widely used in cough drops. It increases cardiac action; an emulsion made by shaking up equal parts of the oil and powdered gum-arabic with water has been used as a urethral injection, in croup and spasmodic throat troubles, the oil may be freely applied externally, the oil is an ingredient of catheder oil, used for sterilizing and lubricating urethral catheters. The industrial oils containing terpenes, which are used for flotation purposes in mining operations. The cosmetic industry uses it as a fragrance component in soaps, detergents, air fresheners, bath oils, and perfumes. Eucalyptus hybrid 'Mysore' is a promising source of pinenes, which are used in synthetic camphor, pine oil, terpineol, and in dry cleaning fluids, solvents, and cheap deodorants.

MATERIALS AND METHODS

In this study thirty pregnant albino Wistar female rats(2-3 months of age, 200-250 gm of weight) were divided into two groups: Control group: consisted of 15 pregnant rats, not subjected to the synthetic eucalyptol oil inhalation vapour but subjected to boiling water inhalation vapour; and experimental group: consisted of 15 pregnant rats, subjected to the synthetic eucalyptol oil inhalation vapour(120 µl eucalyptol oil in 250 ml boiling water); for half an hour from day zero of gestation and for one week. The embryos of rat were obtained at different period of gestation. The embryos at 16,18 day of intrauterine life and one day old were histologically studied for the development of palate and molar tooth germ, sagittal sections through the head of the embryos which were separated from the body. The specimens were prepared for processing and staining with haematoxylin and eosin, and examined under light microscope.

RESULTS

This study demonstrated a significant reduction in the weight measurement of embryos at 18th day intrauterine life of experimental group as shown in (figure 1) in comparison to control. Rat embryo of 16 days intrauterine life subjected to the vapour synthetic eucalyptol oil as shown in (figure 3): showed high-elevated tongue filled the space of the oral cavity and lie in between palatal shelves. Palatal shelves seemed to grow horizontally not fused yet. Nasal septum was detected away from the palatal shelves. Sagittal sections through the upper and lower jaws showed thickened of the oral epithelium. Rat embryo of 18 days intrauterine life subjected to the vapour synthetic eucalyptol oil as shown in (figure 6): showed horseshoe–shaped dental arch with presences of primodium of tooth germ along the arch. High magnification power view showed tooth germ in bud stage.

Rat embryo of one day old subjected to the vapour synthetic eucalyptol oil showed apposition of hard tissue. Polarized ameloblast showed tomes' process facing the developing enamel while odontoblast cells showed to be not well be polarized and not well be arranged faced the developing dentin as shown in (figures 4,5). The predentin showed to be wide, calcosphere can be detected.

Figure 1: Eucalyptol effect on the weight of 18th day rat embryo
Figure 2: A view for rat embryo of one day old (control group) showing tooth germ in advance bell stage, dentine (D), odontoblast (OD), enamel (E), predentin (PD), pulp (P), cervical loop (CL) (HaE ×100).

Figure 3: Microphotograph view for rat embryo of 16-days intrauterine life with synthetic eucalyptol oil vapour, showing palatine shelf (PS), nasal septum (NS), tongue (T) high elevated (HaE ×25).

Figure 4: Tooth germ of upper jaw of subjected rat embryo of one day old with synthetic eucalyptol oil vapour, showing dental papilla (DP), dentine (D) and enamel (E) (HaE ×25).
DISCUSSION

The present study is concerned with tooth development and its relation to the synthetic eucalyptol oil vapour exposure, extending from early sign of developmental stages to the completion of coronal tooth formation including all the consequences of dentinogenesis and amelogenesis, therefore the design of research was selected:

First: pregnant rat from day zero (time of gestation) to expose it to the synthetic eucalyptol oil vapour.

Second: To follow up the effect of the synthetic eucalyptol oil vapour on embryo and specifically to the tooth germ of embryo and its related structure coincide with the age of embryo.

The research studied rat embryo at age 16 day intrauterine life as it illustrated normal tooth development regulated by inductive interactions between cells of the ectodermal lining of the first branchial arch and the underlying mesenchyme. Development begins with a thickening of the oral epithelium, which grows into the mesenchyme forming a bud and inducing the condensation of mesenchymal cells.

At 18 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 bell stage. The late bell stage concerned in the rat embryo of one day old (new born) showing tooth specific cells formed from dental papilla nearest to the epithelium differentiated cells into odontoblast and ameloblast, respectively. In the present study the cap stage has been shown to be a particularly vulnerable stage of the tooth development. As there is a little differences in the time of the tooth development for the first and second molars of the rat and to be precise in the detecting of any variation which illustrates the effect of eucalyptol we selected first and second molars of the rat only and in addition it is more easily to be approach for histological findings.

In all literature studied for eucalyptol effect or its related family on tooth development or other parts of the body (liver, lung....etc). Using of it either orally (oral intubation), capsules, intraperitoneal injection, intravenous dose and intravenous injection for in vivo study and for in vitro study as addition of certain dose to the incubated media which differs from our methodology in using eucalyptol oil as a vapour in pregnant rat. The projection of the idea related to the use of eucalyptol oil vapour in our country for treatment of nasal blockage. Therefore no

Figure 5: High magnification view showed odontoblast cells (OD), dentin (D), enamel (E), ameloblast cells (AB), with Tomes’ process (TP) for subjected rat embryo of one day old with synthetic eucalyptol oil vapour. (HαE ×200).

Figure 6: Low power magnification view for subjected rat embryo of 18-days intrauterine life to synthetic eucalyptol oil vapour showing dental lamina (DL) cover the ridge (upper), tongue (T), (HαE ×25).
research was found to be close to the present work and no work to illustrate the effect of the use of eucalyptol oil vapour by pregnant women on their embryos.

At first time the pregnant rat subjected to 200 µl of eucalyptol oil in 250 ml boiling water (vapour) for half an hour exposure showed abortion after 7-10 days, others died and because of it is an experimental research we reduced the vapour dose into 120 µl microliter of eucalyptol oil in 250 ml boiling water (vapour) for half an hour duration. The results showed reduction in the weight of the embryos at 18th day intrauterine life in comparison to the control ones and it is in agreement with (12) who used related family of the eucalyptol (2,3,7,8-tetrachlorodibenzo-p-dioxin) for adult male rats and they found a reduction in the weight with significant difference in skull size of the experimental rats in comparison to the control one.

**Histological Findings**

The results of the present study showed retardation in the palate and tooth development for experimental group in comparison to the control. It illustrates thickening of the oral epithelium only without detection of tooth germ buds at age 16 day intrauterine life and palatal shelves showed failure of fusion. It supposed that there is a retardation in the metabolic activity if we link with the clinical observation which include reduction in the body weight and the eucalyptol oil as a chemical agent is ubiquitous environmental contaminants that has many adverse biological effects. Their persistence and accumulation in tissue and in the food chain may result in metabolic disturbances and act as a developmental toxicant (13). The detailed mechanisms underlying the spectrum of toxic responses and metabolic retardation elicited by the eucalyptol oil are poorly understood and need for more researches. At 18 day intrauterine life the experimental embryos rat showed tooth germs in bud stage, transited bud to cap stage rather than bell stage which detected in control groups. Retardation in the tooth development at that time suggested arrest of cell differentiation and may be related to poor interaction between mesenchymal cells and epithelial cells and to biological signals that aids in cell differentiation.

Tooth development is genetically strictly controlled but susceptible to environmental disturbances. Among chemicals that have been found to interfere with tooth development in clinical and experimental studies are environmental toxicants such as dioxin and nonhalogenated polycyclic aromatic hydrocarbons as well as fluoride and certain drugs. The effects are not only depend upon the chemical concerned and the dose concentration but also the stage of the tooth development at the time of exposure (14,15). At one day old rat control group showed well apposition of hard tissue enamel and dentin including maturation and mineralization process respectively. Because of the dental hard tissues are not replaced once they have been formed. Tooth seemed to be an informative organ model for studying abnormal mineralized tissue formation and eucalyptol vapour has been shown to have adverse effects on odontoblast and ameloblast. Morphogenesis of ameloblast of experimental group like control once they were polarized, elongated "showing Tomes’ process but enamel showed a defect in a maturation and could be explained that eucalyptol effect ameloblast function and their differentiated stage specifically the maturative stage. For odontoblast cell of experimental group showed morphological change with functional disturbances in comparison to the control. Wide predentin and its border to be mineralized dentin was markedly globular. The results are in line with (13) findings who showed that lactational exposure of rats to 2,3,7,8 tetrachlorodibenzo-p-dioxin interfere with enamel maturation and retards dentin mineralization and they suggested that chemical agent arrests the degradation and matrix proteins or removal of enamel. While for dentin, they found wide predentin and related that to failure of implementation of mineralization or to failure of the conversion of predentin to dentin which involves not only cellular activities but also to structural and compositional changes in the collagenous matrix (16). The line of this study is concerning the effects of eucalyptol vapour on the embryonic tooth germ showed to involve the inductive interactions between epithelial and mesenchymal cells and extend to the dentinogenesis and amelogenesis process.

**REFERENCES**


2. Sfara V, Zerba EN, Alzogaray RA. "Fumigant Insecticidal Activity and Repellent Effect of Five Essential Oils and Seven Monoterpenes on First-Instar


