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Quantification Of Release Of Formaldehyde From The Root Tip.

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ABSTRACT

Formaldehyde containing medications have been used for root canal treatment for many years in endodontics. The normal formaldehyde levels in humans is 2-3gm/kg. A formaldehyde compound, has evolved as the preferred drug for routine endodontic procedures when effective anesthesia could not be obtained. The increase in the use of formaldehyde has been complicated by the introduction of paraformaldehyde pastes for devitalizing the root canal. Formaldehyde, an effective alkylating agent, its action is believed to be due to the release of vapours which act as a antibacterial agent. Formaldehyde applied to vital pulp tissue is absorbed readily into the systemic circulation and distributed throughout the body. A portion of the absorbed formaldehyde is metabolized and excreted by the kidney and lungs. The remaining formaldehyde is tissue bound with the liver, kidney and has known to be of toxic mutagenic and carcinogenic potential.

The paper outlines the quantification of formaldehyde released from the root tip of extracted teeth following the application of the devitalizer on the roof of the pulp chamber using high performance liquid chromatography method and addresses the issue of carcinogenicity associated with formaldehyde.

Keywords: paraformaldehyde, devitalizer, high performance liquid chromatography, carcinogenicity

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INTRODUCTION

The release of formaldehyde is a widely known effect that occurs with many materials used in dentistry. [1]

It is important to understand the difference between formaldehyde gas and formaldehyde solution in order to avoid confusion. Formaldehyde is a flammable gas with a pungent and strong odour. [1]

It is highly soluble in water (up to 55%). Most formaldehyde is sold as aqueous solutions, known as formalin, containing 30–50% formaldehyde with methanol as a stabilizer to prevent it polymerizing into a solid form. Formaldehyde solution is a clear colourless liquid also with a pungent and irritating odour . The chemical formula of formaldehyde is HCHO (or CH2O). [1]

Formaldehyde causes fixation of the pulpal tissue through coagulation necrosis which denatures the protein of the cells and inactivates the oxidative enzymes in the pulp tissue.[2]

In endodontics, patients are exposed to formaldehyde through the use of several endodontic materials that such as N2 paste, Endomethasone, Riebler's paste, and SPAD which have paraformaldehyde levels between 4 and 8%. Other materials such as the epoxy resin cement, for example, AH26 and AH Plus do not contain formaldehyde as an ingredient, but they release minimal levels of formaldehyde during their setting reaction. [3]

The formaldehyde release peaks after 2 days and then slowly decrease for a maximum period of 2 weeks.[3]

Concerns regarding formaldehyde release are based on its known properties as an irritant as well as concerns that it may be a carcinogen. There is controversy as to the risk that formaldehyde presents as a carcinogen, and the possibility that it is a human carcinogen is impossible to exclude formally (Sweetman 2011) even though formaldehyde is not a direct genotoxic agent at sites distant to the portal of entry (nose, oral cavity). [4]

There is no current evidence of harm in humans from the short-term exposures used for formocresol pulpotomies (i.e. minutes), and the doses from epoxy resins in root canal fillings that are at least 40-fold lower than those normally ingested or present in the circulation. (Sue Seale 2010).

This same confusion is evident in a paper from Lewis et al which claims that recently formaldehyde was strongly associated with leukaemia whilst generally accepted as a direct cause of nasopharyngeal cancer'.

World Health Organization (WHO) estimated daily intake of formaldehyde for an adult is about 10.55 mg day^{-1}, comprising 9.4 mg day^{-1} from food, 1 mg day^{-1} from inhalation and 0.15 mg day^{-1} from water. [5]

The American Association of Endodontists issued a position paper on the use of formaldehyde- and paraformaldehyde- containing materials in which they recommended that they should not be used during endodontic treatment due to their toxicity and carcinogenicity (AAE 2013). They stated that the damage from paraformaldehyde-containing filling materials and sealers is not necessarily confined to tissues near the root canal. The active ingredients of these filling materials and sealers have been found to travel throughout the body and have been shown to infiltrate the blood, lymph nodes, adrenal glands, kidney, spleen, liver, and brain. [6]

The International Agency for Research on Cancer (IARC) has classified formaldehyde as 'carcinogenic to humans' (Cogliano et al. 2005), although Marsh et al. (2010) later showed that some of the studies on which this IARC classification was based had incomplete data sets and striking discrepancies and presented misleading evidence.

The US Occupational Safety and Health Administration (OSHA) and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) both regard formaldehyde as a possible human carcinogen and consider that it can cause cancer in animals at high levels that are not found in the majority of workplaces.

In some countries, the endodontic practice involves the use of devitalizer in the vital tooth mainly during cases of a hot tooth and multiple appointments, which not only leads to fixation of the pulpal tissue but also reduces the pain.

Therefore the aim of the present study was to quantify the release of formaldehyde from the root tip of the extracted teeth through a devitalizer applied on the roof of the pulp chamber using high-performance liquid chromatography method and to address the issue of carcinogenecity associated with formaldehyde.

MATERIALS AND METHODOLOGY

Reagents and solutions

All chemical reagents were of analytical grade.

Chemical reagents used were: Deionized water, devitalizer (Caustinerf Forte- 46% paraformaldehyde) was used were used for preparation of test samples and for preparation of standards for HPLC analysis, while 2,4-dinitrophenylhydrazine (DNPH 97%, Sigma-Aldrich, USA) and sulfuric acid (Vetec, Brazil) were employed in the derivatization reactions to prepare FA-DNPH. Acetonitrile (ACN) was used for recrystallization and purification of the derivatization reagent (DNPH).

Method of collection of data

Selection of Teeth

- **Inclusion Criteria:** Intact Mandibular molars with minimal caries and restoration.
- **Exclusion criteria:** Teeth with extensive caries

Procedure

- Eight extracted mandibular premolars and Eight extracted mandibular molars were used
- Access cavity was prepared using a no 2 Endo access bur
- Premeasured devitalizer (2mg) was placed on the roof of the pulp chamber followed by temporization with ZnOE.

Sample and standard preparation for Formaldehyde analysis

The tooth samples which were filled with 2 mg formaldehyde and immersed in deionized water were considered as samples for formaldehyde release analysis. The samples collected after 1 week and 2 weeks were subjected to FA derivatization reaction with DNPH were accomplished by the following procedure (Rezende et al., 2017). The standard stock solution of formaldehyde was prepared by dissolving appropriate masses of previously weighed in an analytical balance in acetonitrile of HPLC grade (20 mg/L). About 2.5 ml of diluted stock solutions of FA (100 to 2000 µg/L) and sample solution (immersed deionized water) were mixed with 2.5 mL of 0.2 g L^{-1} DNPH solution in ACN, which was vortex-mixed for 2 min and centrifuged at 8000 rpm for 15 min. The pH of the resulting solution was adjusted to 4.0 by the addition of 1.0 mol L-1 sulfuric acid before the addition of DNPH solution. These solutions were cooled to -4 °C overnight to freeze the water content, whereas the organic phase remained liquid. The organic phase was quickly removed from the tube using a micropipette, transferred into a 10.0 mL vials and an equal amount of acetonitrile was added in the samples to make 5ml as that of standards. This resulting solution was filtered through a syringe filter (0.22 µm, 25 mm, Himedia, USA) and transferred into vials for HPLC-UV analysis.

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HPLC analysis and operating conditions

Detection of FA-DNPH complex and their quantification was performed with Shimadzu LC-20AD model high-performance liquid chromatography system (HPLC) (Shimadzu, Japan) using Reverse Phase C18 Shiseido Co., Ltd, Japan CAPCELL PAK C18 MGII S5 column (4.6 mm ID x 250 mm) with photodiode array UV-Vis detector (SPD-M20A).

The FA-DNPH complex equivalent to formaldehyde were identified in samples by optimizing and modifying the method of Ochs et al., 2010. The Acetonitrile (solvent A) and water (v/v) (solvent B) were used as mobile phases under binary gradient mode. The binary gradient of 70: 30 (solvent A: Solvent B) was maintained throughout. The column temperature was 30^0 C and absorbance was measured at 360 nm. The flow rate was maintained at 0.3 mL/min with a sample injection volume of 20 µL. The standard graph was developed using the different concentrations ranging from (100 to 2000 µg/L) of standard Formaldehyde which was converted to FA-DNPH complex for analysis.

FINDINGS

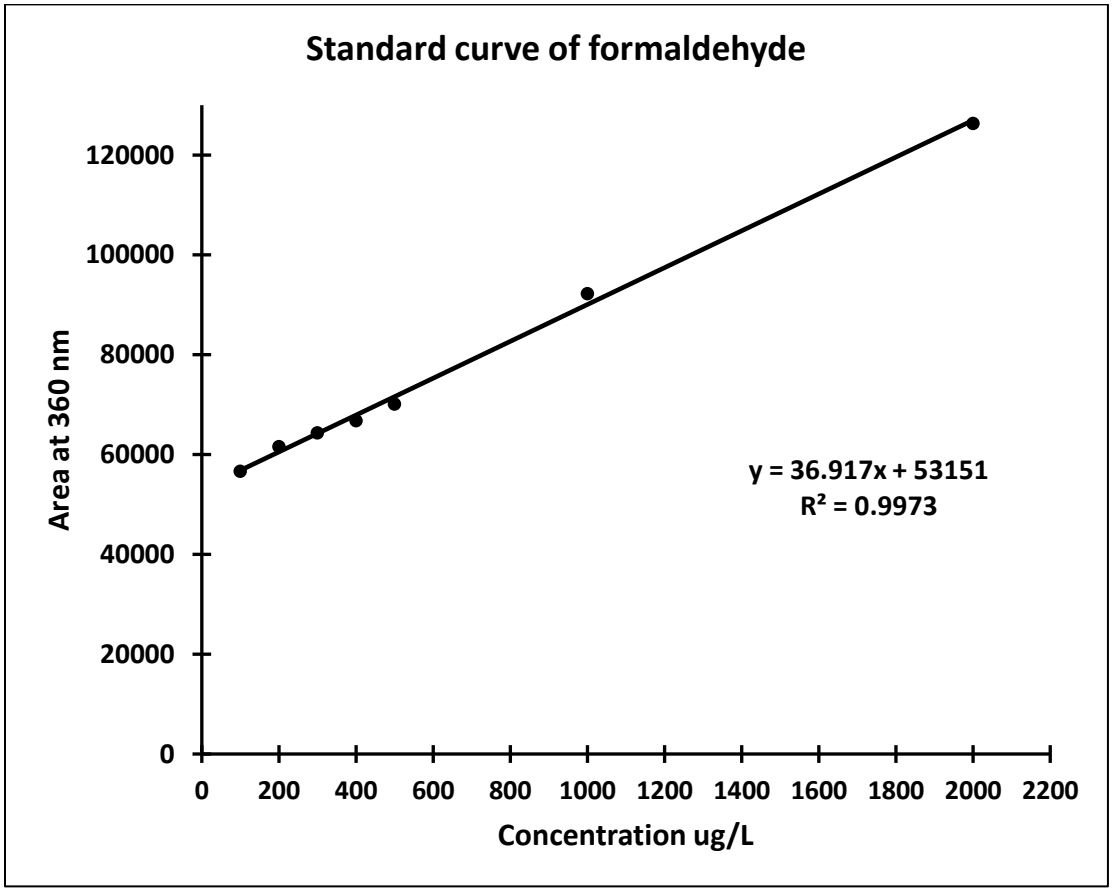

Figure 1: Standard Curve Of Formaldehyde

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1 week				2 week		
Sample	Concentration of formaldehyde detected (µg/ml or mg/L)	Amount released (µg)	% release of formaldehyde from deionized water	Concentration of formaldehyde detected	Amount released	% release of formaldehyde from deionized water
Molar	2.1320 ± *0.0863	21.320	1.07	3.3755 ± * 0.1066	33.755	1.69
Premolar	3.5380 ± *0.0957	35.380	1.77	5.9495 ± *0.0959	59.495	2.97

* - Standard Deviation

Table 1: Concentration Of Formaldehyde From Premolar And Molar In First And Second Week

The present study demonstrated HPLC was able to identify detectable quantities of formaldehyde vapours leaching from the root tip.

This method was confirmed by the linearity if the method obtained by the concentration interval and sample absorbance of the samples.

All the test samples demonstrated minimal diffusion of formaldehyde indicating that dosage released were much lesser than recommended in treatment of permanent teeth which is in accordance to the study done by Dankert et al which involved use of very young teeth and also in accordance to the study done by Kahl et al where formaldehyde released was estimated from 312 blood samples taken before and after the pulpotomies involving children of 2-6yrs age which yielded no detectable formaldehyde above normal baseline physiologic concentrations in any of the blood samples.[7]

Amount of formaldehyde released from molar was 21.32µg on the 7th day which was lesser in comparison with the amount released from the premolar. This could be attributed to the size of the apical foramen which is larger in anterior teeth which increases the permeability of the formaldehyde, whereas in cases of posterior teeth, due to the smaller size of the apical foramen, the larger amount of formaldehyde gets fixed in the dentinal wall and thereby lesser release is seen. This is in accordance to Torneck et al who stated that the tissue reaction to the root canal drugs is influenced by the amount of drugs used, the manner in which the drug is placed, and the size of the apical foramen.[8] [TABLE 1]

Percentage of formaldehyde released from molar was 1.07% on the 7th day which reduced to 0.62% on the 14th day whereas the percentage of formaldehyde released from premolar was 1.77% on the 7th day which reduced to 1.2% on the 14th day. This indicates that the interappointment time should not be more than 1 week since the maximum release was found to be on the 7th day which is in accordance with the study done by Dankert et al.[9] [TABLE 1]

CONCLUSION

In conclusion, it appears that the amount of formaldehyde released during devitalization was at least 1/500 times less than daily intake recommended by WHO after 1 week indicating that devitalizer does not pose any health risks.

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