

EVALUATION OF MUTAGENICITY OF THREE EUGENOL-CONTAINING MATERIALS

Narlan Sumawinata

Department of Conservative Dentistry, Faculty of Dentistry, University of Indonesia

Abstract

Eugenol containing materials are still widely used, both by the lay people or by the dentist. Professionally it is used to relieve dental pain by placing it in the cavity, or as a mixture for temporary filling, temporary cementation, and root canal sealer. Eugenol, however, is also known to be toxic to the tissue, and aflatoxin, a substance known to be mutagenic, has been found in material labeled as clove oil. The purpose of this study was to investigate the safety of these materials, in particular the mutagenicity. The materials tested were imported eugenol material (95.8% eugenol) and local eugenol material (99.5%), both used by dentists, and clove-oil material (6.8% eugenol) used by the lay people. These materials were assayed in Ames test using *Salmonella typhimurium* TA 1535 and TA 1537 strain without S-9 mixture. It was shown that all revertants were below the negative control of the test. It was concluded that neither the eugenol used by the dentist nor the clove oil used for suppressing the dental pain by the lay people showed mutagenicity to *Salmonella typhimurium* TA 1535 and TA 1537 strain.

Key words: eugenol; clove oil; mutagenicity; Ames test

Introduction

Eugenol is a substance which is still widely used in dentistry. It is often used as an anodyne agent, by placing it in the cavity, or as a component of temporary filling and cementation materials. Eugenol is also used frequently as a component of root canal sealer.¹⁻³ Eugenol, however, is known to be toxic to the tissues.⁴⁻⁵ There is a great variability in mutagenicity of eugenol, either according to the tested strain or the method used. Through mouse micronucleus test, it is found that eugenol has mutagenic action to the tissue.⁶ The mutagenic effect of eugenol is also demonstrated by other investigator using *Salmonella* microsome assay.⁷ Other investigators, however, on the different strain of *Salmonella*, found that eugenol has no mutagenic effect⁸ and clove oil (in which eugenol is the major component (70-95%) of this essential oil) has a

component that possesses anti-mutagenic effect to *Salmonella* TA 1535.⁹ It is known that mutagenic effects can be related to various serious diseases, including cancer and birth defects. Therefore, attention should be given to the safety of materials used in dentistry, included eugenol.

Eugenol (1-allyl-3-methoxy-4-hydroxybenzene) is a major component of oil of clove (*Eugenia caryophyllata* Thumb); clove oil contains 70 to 95% eugenol.¹⁰⁻¹¹ Eugenol is also found on betel leaf and marjoram.¹² In Indonesia, people have recognized clove oil since hundreds years ago as a substance to relieve dental pain and as a remedy for diarrhea, flavor for food and perfume as well. The clove cigarette, which contains flower bud of clove mixed with tobacco are widely used by Indonesia smokers. But, on a phytochemical study, aflatoxin, a carcinogenic substance, was found on clove oil marketed in Indonesia.¹³

Based on this finding and considering that eugenol, as a component of root canal filling material, could inadvertently contaminate the periapical tissue, this study was aimed to find out the safety of eugenol-containing materials either used by the dentist (local or imported product) or by the people. This report presents the result of Ames Salmonella assay in which the potential of mutagenicity of the materials were investigated.

Materials and Method

The materials tested were eugenol products used by the dentist and clove oil product used by lay people. Eugenol used by the dentist consisted of imported eugenol material, coded as I (95, 8% eugenol), and local product eugenol, coded as L1 (99, 5% eugenol). local clove oil, used by lay people was coded as L2 (6, 8% eugenol). Dimethyl sulfoxide (DMSO) was used as the solvent of these materials. The strain used was *Salmonella typhimurium* TA 1535 and TA 1537. The assay procedures were performed in four steps. The first step was bacterial isolation, followed by genotype confirmation of the strain, determining the dose, and the mutagenicity test. After the required genotype character of the strain was confirmed, these materials were examined, without S9, at doses of 10 000, 5 000, 2 500, 1250, 1000, 500, 250, 125, and 62, 5 μg per plate. *Salmonella* strain used in this dose-test was TA 100 strain. Due to no viable strain was found on dose more than 1000 μg per plate, the dose used in the mutagenicity test was 62, 5, 125, 250, 500, and 1000 μg per plate. In all tests, negative (solvent) controls (9 AA - 9 aminoacridine) and positive controls with use of known mutagens (ENNG - N-nitro-N-ethyl-N-Nitrosoguanidine) were routinely included. The plates were coded with random numbers before evaluation, and scoring was performed without knowledge of treatment. The mutagenicity of tested materials considered as positive if the number revertants of tested materials were three times of the number of revertants of negative control and no dose-response relationship.¹⁴⁻¹⁵

Result

The result of this study is summarized in Table 1 and Table 2.

Table 1. The number of revertants of *Salmonella typhimurium* TA 1535

spot	NC		PC		Mat	Doses (μg per plate)					
	Conc (μg)	rev	subst	Conc (μg)		Rev	62,5	125	250	500	1000
15-50	15	ENNG	3	209	1	11	14, 13	9	15, 11	8	toxic
						8	14	11	7		
	12	196	L1	11, 12	9	9	11	7	toxic		
				9	12	9	9				
	L2	15, 16	16, 12	7	9	9	toxic				
		10	7	10							

NC = negative control, 9-aminoacridine (9AA); PC = Positive control, N-Nitro-N-ethyl-N-Nitrosoguanidine (ENG); I = imported eugenol; L1 = local eugenol; L2 = local clove oil.

The number of spontaneous revertants per plate for TA 1535 and TA 1537 should be 15 - 50 and 5 - 30 respectively and the number of revertants of positive control for TA 1535 and TA 1537 should be $184,2 \pm 64,9$ and $143,1 \pm 42,7$ respectively (Nohmi, 1989). The results showed that revertants of the negative and positive control controls were within the range, either for TA 1535 (Table 1) or 1537 (Table 2).

Table 2. Mutagenicity of tested materials to *Salmonella typhimurium* TA 1535

spot	NC		PC		Mat	Doses (μg per plate)					
	Conc (μg)	rev	subst	Conc (μg)		Rev	62,5	125	250	500	1000
15-50	15	ENNG	3	209	1	11	14, 13	9	15, 11	8	toxic
						8	14	11	7		
	12	196	L1	11, 12	9	9	11	7	toxic		
				9	12	9	9				
	L2	15, 16	16, 12	7	9	9	toxic				
		10	7	10							

NC = negative control, 9-aminoacridine (9AA); PC = Positive control, N-Nitro-N-ethyl-N-Nitrosoguanidine (ENG); I = imported eugenol; L1 = local eugenol; L2 = local clove oil.

Discussion

The safety of materials used in dentistry and is frequently place in direct contact with living tissue of the oral cavity is a paramount important. Although many of them have been used successfully for a number of years, advances in research, however, have resulted in concern for evaluation and control of the safety of dental materials. The fact that some currently commercial clove oil contained aflatoxin, a carcinogenic substance, albeit in small concentration, highlighted the importance to investigate the safety of materials. The safety test for a substance should include toxicity, teratogenicity, mutagenicity, and carcinogenicity.¹⁴⁻¹⁵ The knowledge of mutagenicity of a material is beneficial in preventing the occurrence of the more severe aftermath. The positive result in a mutagenicity assay is indicative of potential risk to human health because

mutagenesis can relate to life-threatening disease, including cancer.¹⁶ A dental material with mutagenic effect should not be used for treatment or restorative purposes.

The material tested consisted of one imported branded-eugenol, one local branded-eugenol, and one local branded-clove oil. The first two materials usually used by the dentist, while the latter is used by the lay people to reduce dental pain. The local branded-eugenol contain more eugenol than the imported one (99, 5 and 95, 8 % eugenol respectively) while the local branded-clove oil contain only 6, 8% eugenol. None of the three materials tested, however, was exceeding the number of revertants considered to be mutagenic and the dose-response reactivity was not significant. Hence the three tested materials were not mutagenic to *Salmonella typhimurium* TA 1535 and TA 1537 strain. These strains were chosen because no information of the mutagenicity of eugenol to this strain was available.

The present study will enrich data or information concerning the safety of eugenol-containing materials. Previous investigation indicated that eugenol was not mutagenic to *Salmonella typhimurium* TA 98 and TA 100.⁸⁻¹² Because mutagenicity is suggested to further result in carcinogenicity, it was worthy to mention the investigation of Francis which demonstrated that eugenol could suppress aflatoxin, a carcinogenic substance frequently found on Indonesian food.²⁰ On the other hand, Woolverton found that eugenol was a mutagenic substance; he performed his test with the micronucleus test.⁶ The extract of eugenol was also found to be mutagenic to *Salmonella typhimurium* TA 102 strain⁷ and Jeng and his co-workers demonstrated that oral cancer was strongly correlated to chewing bettle quid; bettle quid contains essential oil, the majority of it is eugenol.²² Conversely, Abraham, on mouse bone marrow micronucleus test, had found that eugenol and trans anethole was not genototoxic.²¹ Miyazawa, found that clove oil contained a component that possesses anti-mutagenic effect to *Salmonella* TA 1535.⁹

The present results, with similar strain of *Salmonella typhimurium*, were in accordance with the assay of root canal materials containing eugenol and also known to release formaldehyde,¹⁷ a substance considered to be mutagenic^{18,19} It was an interesting phenomenon, because some researches had suggested not to use materials containing formaldehyde. Eugenol, from clove, however, was demonstrated to have antimutagenic activity,²⁰ and Amonkar and his colleagues also demonstrated that

phenolic compound (eugenol is the major component) of betel leaf had similar activity.¹² Besides its anti-inflammatory and antioxidant activity, Aggarwal and Shisodia demonstrated that eugenol and isoeugenol on clove could suppress NFκB activation by suppressing IκBa degradation.²²⁻²³ NFκB is an abbreviation of Nuclear Factor-kappa Beta. It is a ubiquitous factor that resides in cytoplasm. In cytoplasm, it is bound to its inhibitor, IκB, and when activated it is translocated to nucleus and can induce the expression of COX-2 (inducible cyclo-oxygenase). Injurious and inflammatory stimuli, such as free radicals and Il-1 could release NFκB from its inhibitor (IκB).²⁴

It was demonstrated that the tested materials, either materials containing high concentration of eugenol (95 – 99%) or the lower one (6, 8%) were not mutagenic to *Salmonella typhimurium* strain TA 1535 and TA 1537. Considering that still many controversies concerning the mutagenicity and there is a battery of the mutagenicity test to be performed, it should be noted that the further investigation is still needed.

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