# The cytotoxic effect of different intracanal medicaments

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## ABSTRACT

**Background:** Different intracanal medicaments are being used in endodontics but little attention is paid to their cytotoxicity. The aim of this research was to assess the cytotoxic effect of the traditionally used intracanal medicaments and compare them with 2% acetic acid.

**Materials and Methods:** Different intracanal medicaments and 2% acetic acid were inoculated in the tibia bone of white albino rabbits for 3, 7, 14 and 28 days. After the incubation time, the animals were sacrificed and the inoculated areas were assessed histopathologically.

**Results:** Two percent acetic acid induced an inflammatory reaction comparable to that of normal saline, and calcium hydroxide that subsided after 14 days. The formaldehyde containing agents, and CMCP induced a more severe inflammatory reaction, with tricresol formalin showing a reaction even after 28 days.

**Conclusions:** Two percent acetic acid was more biocompatible than the other tested root canal medicaments.
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## INTRODUCTION

The root canals are connected with the surrounding tissue via the main root canal, lateral, accessory canals, and dentinal tubules. The use of any medicament in the root canal might endanger the surrounding tissues, therefore the cytotoxicity of these medicaments should be as low as possible.

All the intracanal medicaments have certain degrees of cytotoxicity to the surrounding living tissues (1). The formaldehyde containing medicaments are very toxic to the periradicular tissues and can convert the pulp tissues to be an antigen that may trigger an antibody – antigen reaction (2).

CMCP triggers an inflammatory reaction by binding to the protein and lipids of cell membrane causing their disruption (3). CMCP's cytotoxicity is less than that of tricresol formalin (4).

Calcium hydroxide induce a mild-moderate inflamma[1]tion this subsides after 1-3 months (5).

Acetic acid is being examined to be a possible intracanal medicament and has been used in low concentrations in treating certain diseases without any serious cytotoxic effects (6).

## MATERIALS AND METHODS

Twenty white albino rabbits were used for this study. Each rabbit was injected with 3 ml Ketamine (Rotex), and 0.5 ml Xylesin (Rotex), intra muscularly in the thigh region to anaesthetize the rabbit generally.

**1) Procedure of the Operation**

After shaving the legs of the rabbit, the tibia bone was exposed by a longitudinal incision. The longitudinal side of the tibia was chosen to be the site of operation because of its accessibility.

Three 1mm holes were drilled in each bone by contrangled hand piece with 2000 rpm. The normal saline was used as a coolant in the drilling procedure.

A sterile paper point piece of 2 mm length was taken from the wide end of a size 60 paper point. It was impregnated by 0.0025 ml of one intracanal medicament, and was placed in a hole. Each leg with 3 holes received one type of medicament. The inoculated holes were blocked by bone wax, then the operation size was sutured.

For each time interval, five animals were sacrificed that had 30 holes which were grouped according to the following:

Group I: Five holes inoculated by sterile normal saline.
Group II: Five holes inoculated by 2% acetic acid.
Group III: Five holes inoculated by tricresol formalin.
Group IV: Five holes inoculated by formocresol.
Group V: Five holes inoculated by CMCP.
Group VI: Five holes inoculated by 50% calcium hydroxide.

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The animals were kept in the animal house for 3, 7, 14 and 28 days, then they were sacrificed.

**2) Procedure of Histopathology**

The tibia bone was sliced to 2 parts by a disc bur to ensure that the formalin enters and fixes the contents of the bone. The bone was placed in 10% freshly mixed buffered formalin and left for 3 days.

The specimens were decalcified by 5% nitric acid, and sectioned to 5μm thickness to be examined under the microscope, according to the degree of inflammation around the site of inoculation of the medicament and the degree of bone healing.

# RESULTS

Clinically, immediately after placement of the medicaments, only tricresol formalin and formocresol showed a brownish discoloration around the hole with a radius of 4 mm.

The Histopathological pictures are presented in figures 1 and 2.

**1) After 3 Days**

Normal saline (control) and 2% acetic acid induced an inflammatory reaction that was predominantly moderate in nature. Marked vascular congestion was seen around the site with aggregation of inflammatory cells. The adipose tissue of the bone marrow was disturbed by the severe vascular congestion.

Tricresol formalin and formocresol induced a mild-moderate inflammatory reaction. There was limited vascular congestion of blood vessels with few neutrophils around the site. CMCP produced a mild-moderate reaction with a marked vascular congestion, circumscribing the site with a thickness of 1 mm. Calcium hydroxide produced a more moderate-severe inflammation reaction with prominent congested areas around the site and between the adipose tissue.

Statistically, the difference between the medicaments was significant at th level of $p<0.05$. The results are displayed in table 1.

**2) After 7 Days**

The inflammatory reaction began to be more selective. Normal saline and 2% acetic acid induced mild reactions with no vascular congestion around the sites. Tricresol formalin induced a severe-moderate inflammatory reaction with 2 mm thick edematous tissue rim around the site. Formocresol's inflammatory reaction was milder than tricresol formalin. CMCP showed a more diverse picture with a range of mild-severe inflammatory reaction, with many chronic inflammatory cells at the periphery of the site. Calcium hydroxide showed milder reaction than the previous medicaments. The results are displayed in table 2. The relation between the groups was seen to be statistically significant at $p < 0.05$.

**3) After 14 Days**

Normal saline, 2% acetic acid, and calcium hydroxide produced a mild inflammatory reaction, with formation of new bony spicules. The inflammatory cells were few in number, and the adipose tissue of the bone marrow was very close to the site. Tricresol formalin and formocresol had a moderate-mild inflammatory reaction, with the former slightly more aggressive. A fibrous capsule encircled the site, and a granulation tissue rim around the site was present that was thicker in sites inoculated with tricresol formalin.

The inflammatory reaction of CMCP was milder than the formaldehyde containing medicaments, with the presence of bone formation. The results are displayed in table 3. The relation between the groups was seen to be statistically significant at $p < 0.01$.

**4) After 28 Days**

The bony infiltration was predominant and the new bone invaded the site in all the groups except for tricresol formation, which had inflammatory cells at the periphery. The relation between the groups was seen to be not statistically significant. The results are displayed in table 4.

# DISCUSSION

Tricresol formalin and formocresol were found to induce a moderate inflammatory reaction after 3 days, whereas the control and 2% acetic acid showed a severe acute inflammatory reaction. This reaction agrees with the results of Straffon and Han who concluded that the fixative action of the formaldehyde on the surrounding structures delays the infiltration of the inflammatory cells through this layer[7].

**Table (1): The inflammatory reaction of the bone of rabbit after inoculation with different intracanal medicaments for 3 days**

| Medicaments | Inflammatory condition(No. of specimens) | | |
|---|---|---|---|
| | Mild | Moderate | Severe |
| Normal saline | 0 | 4 | 1 |
| Acetic acid 2% | 1 | 3 | 1 |
| Tricresol formalin | 3 | 2 | 0 |
| Formocresol | 5 | 0 | 0 |
| CMCP | 3 | 1 | 1 |
| Calcium hydroxide | 1 | 2 | 2 |

$X^2 = 13.710$, d.f. = 5, $p < 0.05$.

**Table (2): The inflammatory reaction of the bone of rabbit after inoculation with different intracanal medicaments for 7 days**

| Medicaments | Inflammatory condition (No. of specimens) | | |
|---|---|---|---|
| | Mild | Moderate | Severe |
| Normal saline | 4 | 1 | 0 |
| cetic acid 2% | 5 | 0 | 0 |
| Tricresol formalin | 0 | 2 | 3 |
| Formocresol | 4 | 1 | 0 |
| CMCP | 1 | 2 | 2 |
| Calcium hydroxide | 5 | 0 | 0 |

X2 = 19.655, d.f. = 5, $p < 0.01$.

**Table (3): The inflammatory reaction of the bone of rabbit after inoculation with different intracanal medicaments for 14 days**

| Medicaments | Inflammatory condition (No. of specimens) | | |
|---|---|---|---|
| | Mild | Moderate | Severe |
| Normal saline | 5 | 0 | 0 |
| Acetic acid 2% | 5 | 0 | 0 |
| Tricresol formalin | 1 | 4 | 0 |
| Formocresol | 2 | 3 | 0 |
| CMCP | 5 | 0 | 0 |
| Calcium hydroxide | 5 | 0 | 0 |

$X^2 = 18.820$, d.f. = 5, $P < 0.01$.

**Table (4): The inflammatory reaction of the bone of rabbit after inoculation with different intracanal medicaments for 28 days**

| Medicaments | Inflammatory condition (No. of specimens) | |
|---|---|---|
| | None | Mild |
| Normal saline | 5 | 0 |
| Acetic acid 2% | 5 | 0 |
| Tricresol formalin | 3 | 2 |
| Formocresol | 5 | 0 |
| CMCP | 5 | 0 |
| Calcium hydroxide | 5 | 0 |

$X^2 = 5.172$, d.f. = 5, N.S.

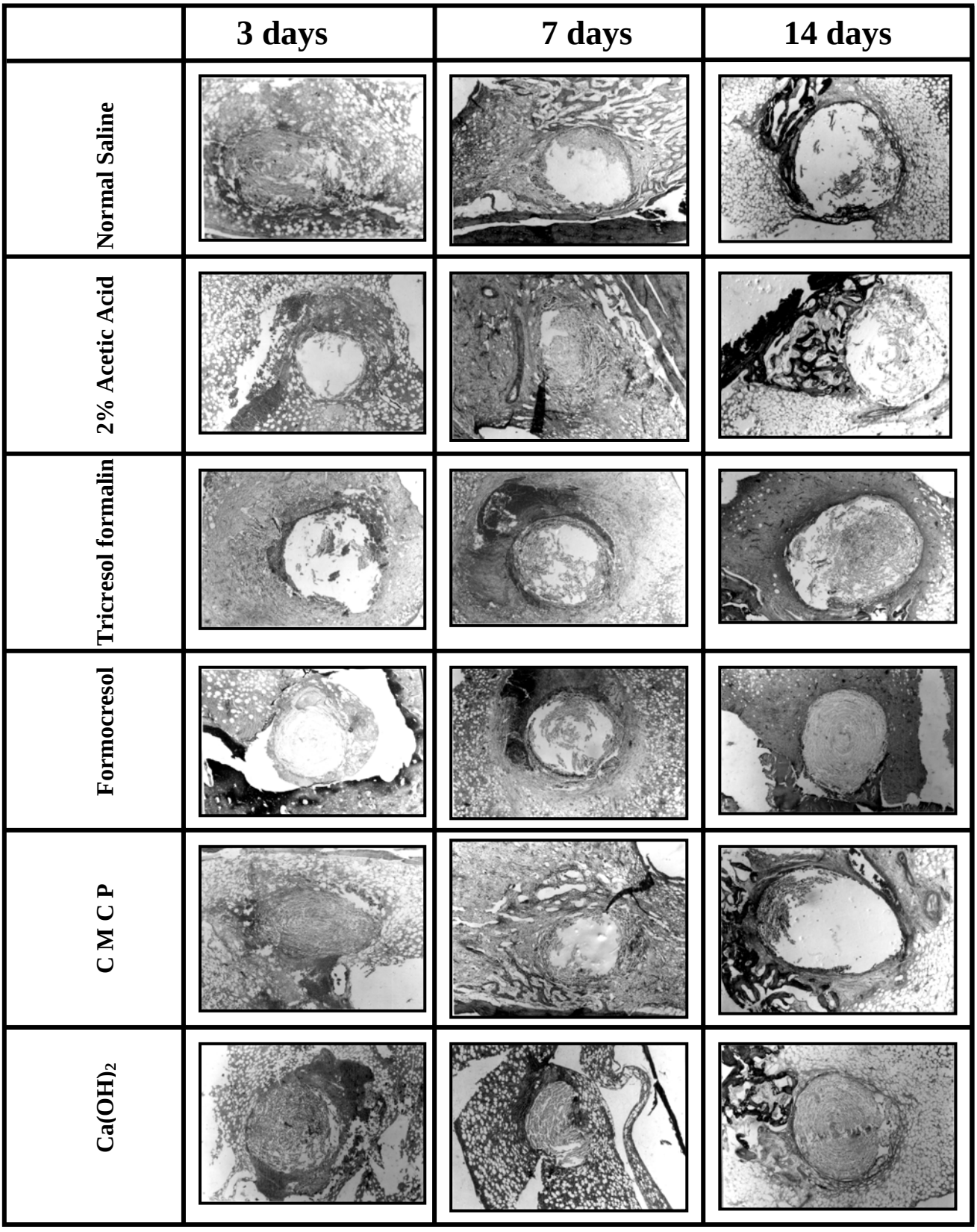
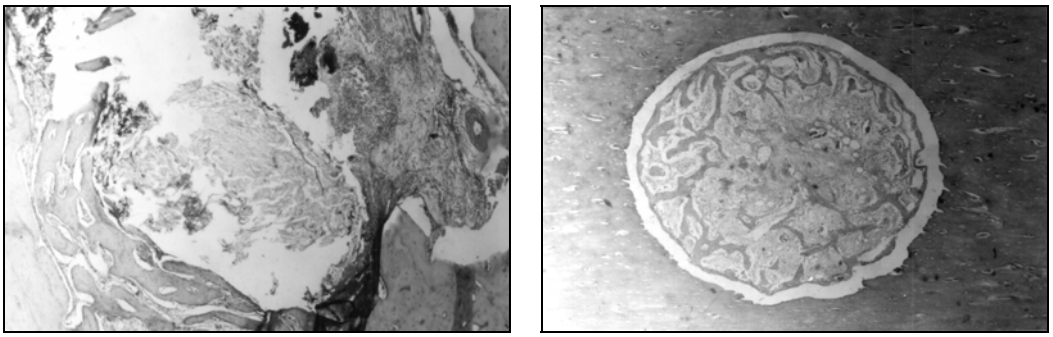



**Figure (1): Histopathological changes after inoculation with different medicaments for different time intervals.**



**Tricresol formalin** **All the other medicaments**

**Figure (2): Histopathological changes after inoculation with different medicaments for 28 days.**

At the seventh and fourteenth day, tricresol formalin and formocresol induced an inflammatory reaction of moderate-mild nature respectively. This result agrees with Powell et al. in 1973, who found a dense inflammatory reaction around a site impregnated with formocresol [8]. The sites treated with 2% acetic acid induced a mild inflammation, probably due to its breakdown by the body's metabolism to $CO_2$ and $H_2O$ [9]. Calcium hydroxide and normal saline showed a similar picture as 2% acetic acid.

The components of CMCP are camphor and monochlorophenol, which are toxic [10]. The inflammatory reaction was milder to that of tricresol formalin and formocresol which is in agreement with Engstrom and Spangberg, who stated that the tricresol formalin is more toxic than camphorated parachlorophenol by 64 times[4]. At the fourteenth day, a connective tissue capsule was evident around the site with a mild inflammatory reaction. This reaction may be due to the rapid dissipation of CMCP to the surrounding tissues, which was confirmed by Fager and Messer who found that 50% of the CMCP was lost after 24 hours and only 1% stayed in the surrounding structures due to its loose complexion with protein and rapid excretion with the urine[11].

At the day 28, all the medicaments experienced no inflammatory reaction with bony formation except for tricresol formalin that may de due to the strong fixative action on the surrounding tissues that is rendered antigenic and induces chronic inflammatory reaction.

It is concluded that two percent acetic acid induced cytotoxicity comparable to that of normal saline, Tricresol formalin and formocresol induced a moderate-severe inflammatory reaction, and CMCP's inflammatory reaction was milder, and finally calcium hydroxide has a mild cytotoxic action.

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