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# Evaluation of Cytotoxic Potential of Various Endodontic Irrigants on Earlichs Ascitic Cells: An Invitro Study

KEYWORDS: cytotoxicity, Earlichs ascitic cell, 2.5% sodium hypochlorite, 2% chlorhexidine, 2% iodine potassium iodide, physiologic saline.

| Dr. Shreema S Shetty | Dr. Darshana Devadiga | Prof(Dr). Mithra .N hegde |
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| Sr.lecturer, Dept of conservative dentistry and endodontics A.B.Shetty institute of dental sciences, Derlakatte, Mangalore | Professor, Dept of conservative dentistry and endodontics A.B.Shetty instituite of dental sciences,derlakatte,mangalore | Senior Professor and Head Dept of conservative dentistry and endodontics, A.B.Shetty institute of dental sciences,derlakatte,mangalore |

**ABSTRACT** *The aim of this study was to investigate the in vitro cytototoxic effects of 2.5% sodium hypochlorite, 2% chlorhexidine ,2% iodine potassium iodide and physiologic saline. Earlichs ascitic cells were used invitro to evaluate the cytotoxicity of the irrigating solutions. The cells were cultured in the peritoneal cavity of Swiss Weister Mice by periodical transplantation of 0.2-0.5ml of sterile suspension in 0.9% saline(containing approx. 5x106 cells/ml). The cells were harvested 10-11 days after transplantation. Results were evaluated calculating means and standard deviations. Data were statistically analyzed by Kruskul-Wallis test ($p<0.005$). all the test irrigants showed variable level of cytotoxicity, with 2.5%sodium hypochlorite having the highest level of toxicity and saline having the least.*

**INTRODUCTION:**

The goal of root canal treatment is to eliminate bacteria,bacterial products and debris from the root canal system,which is accomplished by mechanical action of endodontic instruments and with irrigants. However every root canal system has a complex anatomy with spaces such as webs,fins and anastomoses.[1]Theuse of irrigating solutions is essential to ensure bacterial colony minimization and elimination of necrotic tissue remnants.

Numerous endodontic irrigants are currently in use for endodontics, such as sodium hypochlorite, ethylene diamine tetra acetic acid, chlorhexidine gluconate and physiologic saline. Each irrigant has different properties, and several studies have compared their antimicrobial effect, chemical properties and biocompatibility to establishan ideal solution so asto be used in root canal treatment. It is highly desirable that the chemical agents selected as endodontic irrigants possess favorable properties, such as antimicrobial activity, dissolution of organic tissues and assists in root canal treatment, inducing favorable reaction of periapical tissues, reducing patient discomfort with minimal tissue toxicity and inflammatory response. [2, 3]

A potential complication of irrigation is the forced extrusion of the irrigant and debris through the apex into the periapical area[4]which may occur in vital and non-vital cases in matured teeth and intact apices.[5]Tissue cytotoxicity therefore is a major concern while selecting an endodontic irrigant for therapeutic purposes.

Sodium hypochlorite has been widely recommended as an irrigating solution to aid in the chemo-mechanical debridement of the root canal system because of its dissolving action on pulp tissues and its antimicrobial properties.[6, 7,8, 9.]

Various Studies analyzing the antimicrobial efficacy and residual activity of endodontic irrigating solutions like chlorhexidine, 2% iodine potassium iodide and physiologic salineare known. [10, 11, 12]. However the studies on the cytotoxic potential of these irrigants are very limited.Cell culture tests for evaluating toxicity has been used as a rapid screening assay in first order invitro tests and for assessment of acute cytotoxic potential of the substance. Hence the present study is taken up to evaluate the cytotoxicity of routinely used irrigants on Earlichs ascitic cells.

**AIM AND OBJECTIVE OF THE STUDY:**

To determine and compare the cytotoxic potential of fourendodontic irrigants solution such as 2.5% sodium hypochlorite, 2% chlorhexidine gluconate, 2% iodine potassium iodide, and physiologic saline on cultured Earlichs Ascitic cells.

**MATERIALS AND METHODS:**

2.5% sodium hypochlorite,

2% chlorhexidine gluconate,

2% iodine potassium iodide and

Physiologic saline

The study was conducted at the Central Research Lab,A.B.S.M.I.D.S, Deralakatte, Mangalore, India.The Earlichs ascitic cells were obtained from the department of applied zoology, Mangalore University.Earlichs cells were cultured in the peritoneal cavity of Swiss Weister Mice by periodical transplantation of 0.2-0.5ml of sterile suspension in 0.9% saline(containing approx. $5x10^6$cells/ml).The cells were harvested 10-11 days after transplantation,by aspiration and were sedimented by centrifugation for 15 mins at 1000 xG at room temp.Contaminating erythrocytes,if any were lysed by a brief hypotonic shock. After washing and resedimentation,the cells were resuspended in phosphate buffer solution. Cell counting was done by using a suitable aliquot of harvested and washed cells which were suspended in 0.4% tryphan blue dye prepared in buffer.The suspension was then agitated gently and kept aside for five minutes.An aliquot of the sample after thorough but gentle shaking was charged into haemocytometer and these

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cells were observed under microscope. The cells were then placed in a multi well plates along with the four test irrigants and placed in an incubator at 37$^0$C.Cell viability was assessedusing ELISA readings at the end of 12hrs and 24 hrs.

The samples were divided into four groups

Group 1 -test group

Physiologic saline

Group 2-test group

2.5% sodium hypochlorite

Group 3-test group

2% chlorhexidine gluconate

Group 4- test group

2% iodine potassium iodide

Data was collected at the end of 12and 24 hrs and subjected to statistical analysiswith **Kruskul –**

**Wallis test**

**Results:**
**Table I**

| TEST IRRIGANTS | Mean at 12 hrs | Mean at 24 hrs |
|---|---|---|
| 2.5%NaOCl | 0.132000 | 0.114667 |
| 2%CHX | 0.429000 | 0.750333 |
| 2% IKI | 0.541000 | 0.810667 |
| saline | 1.021000 | 1.553000 |
| P value <0.005-significant | 0.043 | 0.024 |

ELISA Readings of cell viability at the end of 12and 24 hrs. Statistical analysis using Kruskul-Wallis test.

**Table 1** shows the mean values of the test irrigants at the end of 12 hrs with p value-0.043 hence significant difference among the irrigants. The mean values of the test irrigants at the end of 24 hrs with significant p value of 0.024.

Physiologic saline showed the least cytotoxicity followed by 2% IKI, 2% CHX, and the most toxic being the2.5% NaOCl.at the time periods.

**DISCUSSION:**
The debridement of a root canal system with the use of mechanical instruments and irrigants reduces the number of microorganisms, giving a favorable outcome in root canal therapy. The use of cytotoxic irrigants can cause either complications during the course of root canal treatment or may interfere with the repair process. Irrigating solutions should not only be assessed for antibacterial properties but the biological repercussion of their accidental extrusion on host tissue should also be considered. Thus, an ideal irrigant would be one that combines maximum antibacterial activity, and solvent effect on organic and inorganic tissues, with minimal cytotoxic effect onperiapical tissue.[13]

In the present study we have used Earlichs ascitic cell line as these are easily procured and showed results. The number of viable cells inversely represents the level of cytotoxicity of the test materials.

The present investigation was aimed at determining comparative cytotoxicity of 2.5% sodium hypochlorite, 2% iodine potassium iodide, 2% chlorhexidine and physiologic saline on Earlichs ascitic cells under in-vitro conditions. The irrigantsthat were tested showed variable levels of cytotoxicity and was statistically very significant at both 12 hrs and 24 hrs time intervals respectively. 2.5% sodium hypochlorite was most toxic followed by 2% chlorhexidine and 2% iodine potassium iodide, while saline was least cytotoxic. These results are in accordance to a study which measured the cytotoxicity of several endodontic irrigants on cultured gingival cells and found that 2% IKI was significantly less cytotoxic than 2.5% NaOCl.[14].

Sodium hypochlorite is an effective antimicrobial agent on endodontic flora having some tissue dissolving properties and is most commonly used irrigant fluid in root canal preparations. The antibacterial efficacy of the solution is due to the ability to oxidize and hydrolyze cell proteins and osmotically draws fluid out of cells due to its hyper tonicity.The pH of Sodium hypochlorite is between 11-13 and when the hypochlorite comes in contact with tissue proteins nitrogen, formaldehyde and acetaldehyde are released breaking the peptide links with dissolution of the protein, releasing hydrogen from the amino group which gets replaced by chlorineforming chloramines, which has antimicrobial action. As a consequence to these properties, NaOCl is highly toxic at high concentrations and tends to induce tissue irritation on contact.[15].To identify the safest concentration of sodium hypochlorite that is both nontoxic and endodontically effective, several concentrations ranging from 0.25% to 5.25% have been tested and reported in literature[16]. 2.5% NaOCl used in this studyis the optimum concentration recommended in endodontics. [17]

In the present study 2% CHX showed lower cytotoxic potential on Earlichs ascitic cells compared to 2.5% sodium hypochlorite but higher than 2% IKI,these results are in agreement with Gomes –Filho et al. [18] Cytotoxicity of chlorhexidine could be attributed to the inhibition of mitochondrial activity and protein synthesis.[19]

This study suggests that irrigating fluidsmay cause detrimental effects on vital tissues, however the clinician should use appropriate irrigant depending on the clinical situation. Sodium hypochloriteshowed highest toxicity, however its efficiency as one of the ideal root canal irrigant due to excellent antimicrobial efficacy,good tissue dissolving property and lubricant action during instrumentation cannot be provided by any other irrigant at present. 2% iodine potassium iodide and physiologic saline though showed lower levels of cytotoxicity, its other properties such as antimicrobial effect and tissue dissolving property is weak compared to 2.5% sodium hypochlorite.2% chlorhexidine has lower level of cytotoxicity and also has good antibacterial properties, thus can be used in most of the cases including cases with open apex.

**Conclusion**
The present study was assessment and understanding of a cellular event and within limitations of this study,following conclusions were drawn.

2.5% sodium hypochlorite had the most cytotoxic activ-

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ity and saline showed the least cytotoxic potential at the end of 12hrs and 24 hrs time period respectively.Further research will help shed light on other features such as the tissue reactions that occur when irrigants are applied in the root canal.

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