ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 04 | April - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

MICROSCOPIC EVALUATION OF CYTOTOXIC EFFECTS OF VARIOUS ROOT CANAL IRRIGANTS ON HUMAN BLOOD CELLS - AN EX-VIVO STUDY.

Endodontics

C. Sunil Kumar	Professor, Department ofConservative Dentistry And Endodontics C.k.s. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India.
B. Saranya*	Post graduate student,Department of Conservative Dentistry And Endodontics C.K.S. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India. *Corresponding Author
S.Datta Prasad	Prof and Head of the Department, Department of Conservative Dentistry And Endodontics C.K.S. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India.
N.Vamsee Krishna	Associate Professor, Department of Conservative Dentistry And Endodontics C.K.S. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India.
S. Sunil Kumar	Reader,Department of Conservative Dentistry And Endodontics C.K.S. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India.
K.S. Chandra Babu	Reader, Department of Conservative Dentistry And Endodontics C.K.S. Theja Institute of Dental Sciences And Research Renigunta Road,tirupati Chitoor District-517501, Andhra Pradesh, India.

ABSTRACT

Aim: To Elucidate the potential cytotoxicity of 5.25% Sodium Hypochlorite, 2% Chlorhexidine Gluconate, Q-mix, Morinda Tinctoria Extract and Aloe Vera Extract Individually and to compare the same with Normal saline on Human Blood Cells.

Materials and methods:- A 5 ml of Human Blood was drawn from a Human volunteer and collected into Heparinized test tubes. Blood is spun at 1000 rpm for 10minutes in a Centrifuge Machine. After centrifugation of the blood, Plasma is removed and Packed Cell Volume (PCV) of Blood Corpuscles is obtained. 2ml of PCV is added to 8ml of saline.100µL of this solution is added to 80 test tubes each and were divided into 5 experimental groups (NaoCl, CHX, Q-mix, M.Tinctoria, Aloe Vera) with 15 Test tubes in each group and each group is sub divided into 3 groups of 5 Test tubes based on concentration of the Irrigants used i;e 10µL, 30 µL and 50 µL. 1 control group (saline) contains 5 samples. After adding Irrigant to the test tube it is incubated for 3minutes. Peripheral Smears were prepared and stained with Leishman stain. Morphological alterations of blood cells in each slide were evaluated using Oil Immersion Microscope.

Results:- Sodium hypochlorite shows maximum cell changes followed by Q-mix, followed by Chlorhexidine, followed by M.Tinctoria. Aloe Vera showed least Catastrophic changes among all other experimental groups other than Normal Saline which showed no changes.

Conclusion:- This study suggests that these Irrigating solutions do cause detrimental effects on Human Blood Corpuscles. Therefore a great deal of care should be exercised while using Irrigants during Endodontic procedure.

KEYWORDS

INTRODUCTION

Endodontic therapy is primarily based on removal of potentially noxious stimuli from the complex root canal system. Irrigants flush debris from canals and assist in reducing microbial flora of infected canals and helps to dissolve necrotic tissues. The persistence of residual pulp tissue infected dentine or bacteria in the root canal system may be responsible for treatment failure.[1]

Chemo mechanical debridement remains mainstay in the success of root canal treatment. Several Micro computed tomography studies have demonstrated that proportionally large areas of the root-canal will remain untouched by the instruments, emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.[2] It is highly desirable that the endodontic Irrigants possess 4 major properties 1) antimicrobial activity 2) dissolution of organic tissues 3) debridement of canal 4) non-toxicity to periapical tissue. As in both vital and non-vital cases extrusion of Irrigants occur even in teeth with fully formed mature intact apexes, Blunderbuss canals, perforations and improper techniques can also allow solution to permeate into surrounding tissues.[1]

An endodontic irrigant should be non-toxic when it comes in contact with vital tissues and non-caustic to the periodontal tissues. A potential complication of irrigation is the forced extrusion of the irrigant and debris through the apex. Tissue cytotoxicity is therefore a major concern when choosing an endodontic irrigant for root canal treatment. Various methods have been employed to evaluate the cytotoxicity of endodontic Irrigants.

Pashley et al. evaluated the cytotoxicity on red blood corpuscles, **Faria et al**. and **Zhang et al**. evaluated the cytotoxicity on L929 fibroblast and **Barnhart et al.** on gingival fibroblast.[7]

Therefore, the aim of this study was to analyze the cytotoxicity of various volumes of 5.25% Sodium Hypochlorite, 2% Chlorhexidine Gluconate, Q Mix 2 In 1 Solution, Aloe Vera Extract and Morinda Tinctoria by checking for Cellular changes in Blood Cells.

Materials and methodology:-

GROUP I - 5.25% Sodium hypochlorite
GROUP II - 2% Chlorhexidine gluconate
GROUP III – Q-mix solution
GROUP IV - Aloe vera extract
GROUP V - Morinda tinctoria extract
GROUP VI – Control group

- A 10 ml of human blood was drawn from a human volunteer and collected in blood storage test tubes.
- Blood is spun at 1000 rpm for 10minutes in a Centrifuge Machine.
- After centrifugation of the blood, plasma is removed and packed cell volume of blood corpuscles is obtained.
- A volume of 2 ml of packed cell volume is added to 8 ml of saline to increase the volume of blood to 10 ml.
- A volume of 100 µl of this diluted blood corpuscles is added to 80 test tubes and were divided into 5 experimental groups (NaOCl, CHX, Q-mix, M.Tinctoria, Aloe Vera) with 15 Test tubes in each group and each group is sub divided into 3 sub groups based on

volume of the Irrigants used i;e 10µL, 30 µL and 50 µL and each subgroup contains 5 Test tubes. 1 control group (saline) contains 5 samples.

- After adding Irrigant to the test tube it is incubated for 3minutes. Peripheral Smears were prepared and stained with Leishman stain.
- Morphological alterations like **Sickling, Ghost Cells, Cell Lyses, Roulex Formation, Cytoplasmic Vacuolation, Echinocytes, Anisocytosis, Poikilocytosis, Agglutination and Clumping** of blood cells in each slide at volumes of **10 µL, 30 µL, and 50 µl of different irrigants** were evaluated using Oil Immersion Microscope at 100X magnification.

OBSERVATIONS AND RESULTS:-

Data was statistically analyzed using SPSS V24. Descriptive statistics were represented with mean and SD using Kruskal-Wallis Test and Chi Square test were applied to find significance and pairwise comparisons between groups. P< 0.05 was considered as statistically significant.

The smears with blood cells treated with 5.25% sodium hypochlorite and Q-mix revealed Poikilocytosis, Anisocytosis, Irregular clumping, Agglutination and Sickling of few RBC's were also seen. Toxic changes in the white blood cells encompass cytoplasmic vacuolization and often caused cell lysis. Ghost cells resembling fat or oil droplets with colorless spherical membrane are present.

In case of 2% CHX and M.tictoria, peripheral smear examination revealed irregular clumping and lysis of RBC was seen. Smears with Aloe Vera reveals intact surface integrity of RBC and WBC without any lysis and devoid of cytoplasmic vacuolation Echinocytes are seen in the peripheral smear. Smear with RBC suspension when treated with isotonic saline revealed intact RBC AND WBC cell surface without any cytoplasmic vacuolation or disruption.

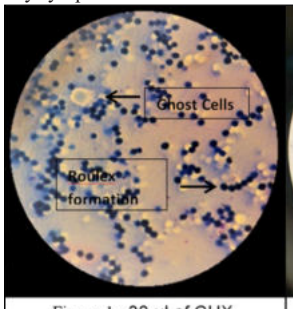

Figure 1: 30 µl of CHX

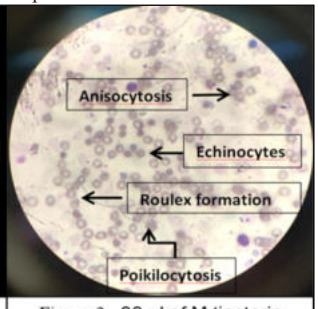

Figure 2: 30 µl of M.tinctoria

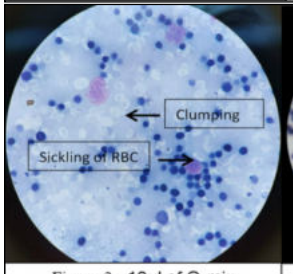

Figure 3: 10µl of Q-mix

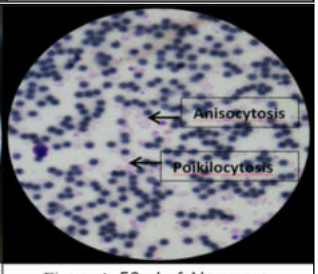

Figure 4: 50 µl of Aloe vera

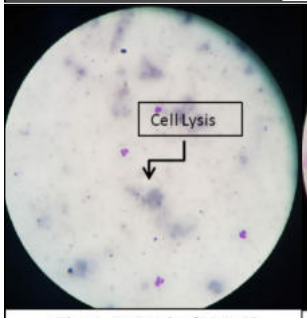

Figure 5 : 50 µl of NaOCl

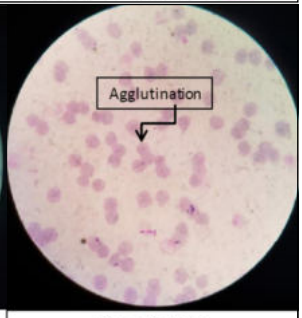

Figure 6: Saline

Table No 1:- Kruskal-wallis Test: Non Parametric Test

	TEST STATISTICS					
	Cytotoxicity At The Concentration 10 UL					
	Naocl	**Chx**	**Qmix**	**Aloevera**	**M.tinctoria**	**Saline**
Asymp. Sig.	0.196	0.002	0	0.422	0.001	0.026
Cytotoxicity At The Concentration 30 UL						
	Naocl	**Chx**	**Qmix**	**Aloevera**	**M.tinctoria**	**Saline**
Asymp. Sig.	0.367	**0.031**	0.247	0.073	0.054	**0.026**
Cytotoxicity At The Concentration 50 UL						
	Naocl	**Chx**	**Qmix**	**Aloevera**	**M.tinctoria**	**Saline**
Asymp. Sig.	**0.003**	0.12	0.321	**0.003**	0.899	**0.026**

Table No 2:- Intra Group Comparasion Of Cellular Changes At Different Concentrations (10ul, 30ul, 50ul)

Test Statistics						
	NOACL	CHX	Q MIX	ALOEVERA	M.TINCTORIA	SALINE
Chi-Square	2.162	1.407	0.566	4.42	1.565	0
Asymp. Sig.	0.339	0.495	0.753	0.11	0.457	0.01
a. Kruskal Wallis Test						
b. Grouping Variable: Group						

DISCUSSION:-

The goal of endodontic therapy is to completely debride the root canal system, preventing the bacterial re-infection. This is necessary in order to create an environment for healing and maintain the healthy periodontium. This eliminates the pain and suffering caused by pulpal inflammation or infection. For disinfecting the complex root canal system, a variety of root canal irrigants have been recommended. Endodontic implications, such as unintended extrusion of these root canal irrigants beyond the apex will generate unusual iatrogenic complications instead of alleviation.[5]

White and Red blood cells were chosen as biological model to evaluate cytotoxic effects as these cells because they can be easily isolated using least invasive procedure. The red blood cell membranes are semi permeable barriers and the osmotic gradient established on either side of membrane causes the fluid to flow into and out of the cells.[4]

The amount of osmotic pressure depends upon the difference between the concentrations of non diffusable ions on each side of the membrane. When the cells are subjected to hypertonic solution, they undergo rapid osmotic efflux of water leading to crenation and finally collapse. On the other hand, in hypotonic solution, the cells swell and lyse liberating the cell constituents into the suspension media which results in morphological alteration. Hence an altered morphological characteristic is considered to be one of the parameter to evaluate the cytotoxic effects.[6]

The use of 5.25% NaOCl for biomechanical preparation of root canals is a clinically acceptable and highly effective procedure. Hypochlorite preparations are sporicidal, virucidal and show far greater tissue dissolving effects on necrotic than on vital tissues. The higher the concentration the better will be the antimicrobial effect and the tissue dissolving capacity. At the same time higher concentration also carries the risk of toxicity and tissue reaction. It is cytotoxic to all cells except heavily keratinized epithelia (Pashley etal 1985). From the present study, NaOCl is most cytotoxic at cellular level compared to other irrigants and it should be used with high caution. The hemolysis and the morphological alteration that occurred might be due to the strong oxidizing effect of NaOCl on the cell membrane rather than osmolysis.[7]

At the volume of 10 µL **(0.196)** and 30 µL **(0.367)** NaOCl shows no statistically significant difference among the observed cell changes because, even at this volumes smears treated with NaOCl reveals all types of cell changes including cell lysis. At a volume of 50 µL (**0.003**) NaOCl shows statistically significant difference among the observed cell changes because at this volume this irrigant causes complete cell lysis of the slide without any other cell changes.

Shreeshail Indi et al done a study on peripheral smear of RBCs treated with 3% sodium hypochlorite revealed **poikilocytosis, anisocytosis**, Irregular clumping, agglutination and sickling of RBC's.

Toxic effects of sodium hypochlorite on cells was explained by **Dian Agustin Wahjuningrum et al**, NaOCl produces hypochlorous acid (HOCL), which is an oxidizing agent acting as a solvent and when it comes in contact with organic tissue, it releases chlorine. However, chlorine can trigger the onset of free radicals, which will increase reactive oxygen species (ROS). In addition, NaOCl has a high pH that triggers the release of hydroxyl ions. This release causes a change in

the integrity of the cytoplasmic membrane leads to cell death[8].

Sodium hypochlorite and Chlorhexidine doesn't have smear layer removal property, EDTA has smear layer removal property but lacks of effective antimicrobial property to enhance the quality of treatment we need both properties. Advances in irrigating solutions develops a new irrigant named as Mix, Q-Mix is a 2-in-1 solution containing a bisbiguanide antimicrobial agent (2% CHX), poly amino carboxylic acid calcium-chelating agent (17% EDTA) and a surface active agent. It has a both properties of chlorhexidine and EDTA. Q Mix was found to be effective in removing the smear layer and also has substantial antimicrobial properties.

At the volume of 10 µL **(0)** Q-mix shows statistically significant difference among the observed cell changes without presence of cell lysis. At the volume of 30 µL **(**0.247**)** and 50 µL (0.321) Q-mix shows no statistically significant difference among the observed cell changes because at this volumes smears treated with Q-mix reveals all types of cell changes including cell lysis.
Q Mix causes death in cells because of its contents CHX and EDTA. CHX increases the osmotic gradient there by liberating haemoglobin. EDTA as a chelating agent it has interaction with endogenous metal inorganic ions in cells which results in the interference with the structure and permeability of cell membranes.

Present study results were in accordance with **AlKahtani Ahmad et al**, The viability of cells exposed to NaOCl was significantly lower than that of cells exposed to Q Mix™ at all time intervals, A high concentration (0.5 mg/ml) of NaOCl caused the vacuolization of the cytoplasm, whereas Q Mix™ at the same concentration produced few alterations in the cell wall and no vacuolization in human bone marrow mesenchymal stem cells.

Chlorhexidine is a cationic bisbiguanide with optimal antimicrobial action ranging from pH 5.5 to 7.0. It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria, bacterial spores, lipophilic virus, yeast and dermatophytes. It seems to act by adsorbing onto the cell wall of the micro organism and causing leakage of intracellular components.[9]

At the volume of 10 µL **(**0.002**)** and 30 µL **(0.031)** CHX shows statistically significant difference among the observed cell changes because at this volumes smears treated with CHX reveals only some types of cell changes. At a volume of 50 µL (0.12) CHX shows no statistically significant difference among the observed cell changes because at this volume this irrigant presents all types of cell changes including cell lysis in the smears.

Cytotoxicity of CHX may be due to the increased osmotic permeability and altered osmotic pressure. This might cause the influx of the fluids and liberation of the haemoglobin out of the cell leading to the formation of ghost cells. The toxicity of Chlorhexidine to human gingival cells showed that the toxic potency of Chlorhexidine is dependent on the length of exposure and the composition of the exposure medium **(Babich et al.1995).**[10]

Present study results were agreed with **Jayalakshmi Pandranki et al** they observed the peripheral smear examination of 2 % Chlorhexidine they revealed poikilocytosis with cytoplasmic vacuolation in the RBC and increased number of ghost cells. Irregular clumping of RBC is also seen. White blood cells with cytoplasmic vacuolation and cell lysis are seen.[5]

As a trend in all life branches to replace harmful chemicals by natural biocompatible alternatives Aloe vera and M.Tinctoria are used as irrigants in the study.

Aloe vera, it is a herb bestowed with therapeutic and cosmetic properties, is a member of the Asphodelaceae family **Bhardwaj et al.** Assessed antibacterial activity of Aloe Vera gel as long as1, 3 and 5 days. Aloe Vera showed good antibacterial activity just at the first day of incubation. They noted that Aloe Vera had 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids which were possible reasons for its antimicrobial action. [11]

At the volume of 10 µL **(**0.422**)** and 30 µL **(**0.073**)** smears treated with Aloevera shows no statistically significant difference among the observed cell changes because at this volumes no cell changes were observed. At a volume of 50 µL (**0.003**) Aloevera shows statistically significant difference among the observed cell changes because at this volume this irrigant shows only mild cell surface changes like Anisocytosis which are reversible.

Morinda tinctoria, it belongs to family – Rubiaceae, Morinda Tinctoria at a concentration of 60 mg/ml has antimicrobial substantivity lasted for about 12 days against Enterococcus faecalis which can be comparable to chlorhexidine efficiency indicating its ability to inhibit the growth of E. faecalis for longer period.[5]

Morinda plant showed the presence of alkaloids, tannins and polyphenols in aqueous and methanol extracts (**Shanthi G, et al)**.

At the volume of 10 µL **(**0.001**)** and 30 µL **(**0.054**)** M.tinctoria shows statistically significant difference among the observed cell changes because at this volumes smears treated with CHX reveals only some types of cell changes. At a volume of 50 µL (0.899) M.tinctoria shows no statistically significant difference among the observed cell changes because at this volume this irrigant presents all types of cell changes including cell lysis in the smears.

The lipoproteinaceous phytochemical constituents of herbal extract, Morinda Tinctoria might lead to the echinocytic transformation of the red blood cells.

Smears treated with saline (0.026) reveals statistically significant difference among the observed cell changes because only mild cell surface changes were observed with this irrigant. Cell changes may occur because of Saline removes the protective coating of the plasma and can crenate the discocyte systematically . But these changes are reversible after few minutes.[12]

CONCLUSION:-

Irrigating Solutions used do cause detrimental effects on the diluted Red Blood Corpuscles, White Blood Cells And Hemoglobin. Sodium Hypochlorite has been proven to be highly cytotoxic and a great deal of care should therefore be exercised while using this during endodontic irrigation.

Within the limitations of this Ex-Vivo Study, Aloevera Extract is Considered to be Least Cytotoxic Compared to 5.25 % Naocl, 2 % Chlorhexidine, Q Mix 2 In 1 Solution, Morinda Tinctoria Extract.

Further investigations should be carried out to assess potential benefit of Aloe vera Extract and Morinda Tinctoria Extract to be used clinically as they are proved to be least cytotoxic compared to other commercially available irrigants.

REFERENCES:-

1. Shetty S, Rai N. Comparison of cytotoxicity of endodontic irrigants at various concentrations: An invitro study.
2. Indi S, Kulkarni S. Cytotoxic Analysis of Herbal Root Canal Irrigants at Cellular Level. Group.;3:0-68.
3. **Shreeshail Indi, Sangeeta Kulkarni. Cytotoxic Analysis of Herbal Root Canal Irrigants at Cellular Level. International Journal of Research and Scientific Innovation-IJRSI vol.3 issue 6, pp.50-54 2016**
4. Shetty KP, Satish SV, Kilaru K, Ponangi KC, Venumuddala VR, Ratnakar P. Comparative evaluation of the cytotoxicity of 5.25% sodium hypochlorite, 2% chlorhexidine and mixture of a tetracycline isomer, an acid and a detergent on human red blood corpuscles: An in-vitro study. Saudi Endodontic Journal. 2014 Jan 1;4(1):1.
5. Pandranki, Jayalakshmi & Rao, V. & Chandrabhatla, Srinivas. (2015). Cytotoxic Analysis of Various Root Canal Irrigants at Cellular Level. British Journal of Medicine and Medical Research. 6. 1052-1062. 10.9734/BJMMR/2015/15507.
6. Jay AWL, Rowlands S. The stages of osmotic hemolysis. J. Physiology.1975;252:817-882.
7. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. Journal of Endodontics.1985;11(12):525-528.
8. Wahjuningrum DA, Elizabeth ME, Puteri FH, Mardiyah AA, Subiyanto A. Cytotoxicity assay of sodium hypochlorite and QMix on cultured human periodontal ligament fibroblast cells. Journal of International Oral Health. 2019 Jul 1;11(4):204.
9. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. International endodontic journal. 2003 Feb;36(2):75-85.
10. Babich H, Wurzburger BJ, Rubin YL, Sinensky MC, Blau L. An in vitro study on the cytotoxicity of chlorhexidine digluconate to human gingival cells. Cell Biol Toxicol. 1995 Apr;11(2):79-88. doi: 10.1007/BF00767493. PMID: 7583874.
11. Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against Enterococcus faecalis: An in vitro study. Journal of conservative dentistry: JCD. 2012 Jul;15(3):293.
12. Gerald Lim HW, Michael Wortis, Ranjan Mukhopadhyay. Stomatocyte–discocyte–echinocyte sequence of the Human red blood cell: Evidence for the bilayer– couple hypothesis from membrane mechanics. Proc. Natl. Acad. Sci. 2002;99(26): 16766–16769.