Cytotoxic Analysis of Herbal Root Canal Irrigants at Cellular Level

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Abstract:

Aim: To evaluate and compare the cytotoxic effects of commercially available root canal irrigant sodium hypochlorite and herbal extracts Turmeric & Neem.

Methodology: Sixty samples with 2ml of RBC suspension was randomly assigned to four groups. 3% Sodium hypochlorite, 0.25% Hydro-Alcoholic solution of Turmeric and 7.5% Hydro-Alcoholic solution of Neem and they were tested on RBC suspension. Normal saline was selected as control. Peripheral smear were prepared to assess the morphological abnormalities of viable cells. After centrifugation of each test tube, the supernated volume was estimated for haemoglobin concentration representing cytotoxicity.

Results: Cytotoxicity varies in the following order: 3% Sodium hypochlorite > 0.25% Hydro-Alcoholic solution of Turmeric > 7.5% Hydro-Alcoholic solution of Neem. Results showed that statistically significant difference exists between cytotoxicity of tested irrigating solutions.

Conclusion: Considering the undesirable effects of the conventional root canal irrigants, herbal extracts could be an alternative root canal irrigant with least toxicity.

Keywords: Sodium hypochlorite; turmeric; neem; cytotoxicity; red blood cells.

I. INTRODUCTION

Removal of vital and necrotic remnants of pulp tissues, microorganisms, and microbial toxins from the root canal system is essential for endodontic success. Chemomechanical debridement remains mainstay in the success of root canal treatment.[1]

Several studies using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main 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]

Irrigation has a central role in endodontic treatment. Several irrigating solutions also have cytotoxic potential, and they may cause severe pain if they gain access into the periapical tissues.[3]

Sodium hypochlorite (NaOCl) is the most popular irrigating solution used in concentrations between 0.5% and 6%. It is a potent antimicrobial agent, killing most bacteria instantly on direct contact. The main drawbacks of NaOCl is its cytotoxicity and caustic nature[4].

Because of the increased antibiotic resistance, toxic and harmful side effects of few common antibacterial irrigants, there is a need for alternative agents which are affordable, non-toxic and effective[5].

Turmeric (Curcuma Longa) and Neem (Azadirachta indica A) are natural medicament with a wide spectrum of biologic actions which include anti-inflammatory, antioxidant, anticoagulant, anticarcinogenic, antimutagenic, antidiabetic, antibacterial and antifungal activities.[6-7]

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 fibroblasts and Barnhart et al. on gingival fibroblasts.[8-11].

Therefore, the aim of this study was to analyze the cytotoxicity of various irrigants, 3% sodium hypochlorite, 0.25% Hydro-Alcoholic solution of Turmeric and 7.5% Hydro-Alcoholic solution of Neem by checking for hemolysis of human red blood corpuscles.

II. MATERIALS AND METHODS

Red blood cells are very sensitive to osmotic challenge by hypo- or hypertonic solutions which cause hemolysis and they are readily available for analysis of intracellular content, and hemoglobin.[12].

In this study 2 parameters were analysed.

1) Haematocrit value (Hb%)
2) Cellular changes in RBCs

III. PREPARATION OF RBC SUSPENSION

Red blood cells were selected to evaluate the cytotoxicity. Fresh blood from human volunteers was drawn into EDTA
containers, spun at 1000 rpm for 10 minutes, the plasma was discarded and the packed cell volume obtained was washed twice in Dulbecco’s phosphate buffered saline by centrifugation. The final hematocrit of the RBC suspension was adjusted to 45%.

IV. CYTOTOXIC ANALYSIS

3% sodium hypochlorite (NaOCl) and herbal extracts were tested against viable cells for cytotoxic evaluation. The solutions were grouped as follows:

- Group 1 - 3% Sodium hypochlorite
- Group 2 - 0.25% Hydro-Alcoholic solution of Turmeric
- Group 3 - 7.5% Hydro-Alcoholic solution of Neem
- Control group - Saline

100 μl of each irrigants was added to 2 ml of the diluted RBC suspension in individual test tubes separately. After incubating for 3min, Morphological alterations in the RBC were evaluated with Leishman’s stain.

Tubes were then centrifuged at 1000 rpm for 10 min and the supernated volume obtained was subjected to haemoglobin estimation measured by hematology analyzer (Sysmex automated hematology analyzer KX-21N) which uses noncyanide hemoglobin analysis method[13].

The readings obtained were tabulated. The data obtained was subjected to statistical analysis using ANOVA and student T test.

V. RESULTS

Because of hemolysis, the mean increase in the hemoglobin concentration was 0.68 gm% for NaOCl, 1.96 gm% for turmeric and 3.04 gm% for neem. Almost there is no hemolysis with saline. The results obtained were statistically highly significant with a p value of 0.05 and F ratio of 66.93.

All groups showed significant difference in the cytotoxic effects when compared with saline.

<table>
<thead>
<tr>
<th></th>
<th>GROUP I (NaOCl)</th>
<th>GROUP II (Turmeric)</th>
<th>GROUP III (Neem)</th>
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<tbody>
<tr>
<td>MEAN</td>
<td>0.68</td>
<td>1.96</td>
<td>3.04</td>
</tr>
<tr>
<td>SD</td>
<td>0.15</td>
<td>0.18</td>
<td>0.38</td>
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<tr>
<td>F VALUE</td>
<td>66.93 (&gt;1.96 FOR P=0.05)</td>
<td>There is significant difference between three groups</td>
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</tbody>
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Table I - Mean and Standard deviation (SD) of difference in Hb (%) for three groups with respect to control group. Comparison between three groups by one way Anova (F value)

<table>
<thead>
<tr>
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<th>T value</th>
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<tr>
<td>Group I and Group II</td>
<td>7.13</td>
</tr>
<tr>
<td>Group I and Group III</td>
<td>21.93</td>
</tr>
<tr>
<td>Group II and Group III</td>
<td>2.86</td>
</tr>
</tbody>
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All t values >1.96 for p=0.05, shows significant difference two groups

Table II - Comparison between Groups by Student’s T test

Fig. 1. Means of differences in Hb% for three groups with respect to control group

Fig. 2. Saline

Fig. 3. 3% NaOCl
Fig 4. 3% NaOCl

Fig 5. 3% NaOCl

Fig 6. 3% NaOCl

Fig 7. 0.25% Turmeric

Fig 8. 0.25% Turmeric

Fig 9. 7.5% Neem

Fig 10. 7.5% Neem

Labelling: - a) Intact RBC b) Clumping of RBC c) Echinocytes d) Intact WBC e) Ghost cells f) Rouleaux g) Cytoplasmic vaculations h) Anisocytosis i) sickling j) lysis of RBC k) Discocytes
VI. MICROSCOPIC FEATURES

The peripheral smears of the RBC suspension were evaluated under oil immersion microscopy at 100X magnification. The smear with RBC treated with 3% sodium hypochlorite stained after 3 minutes incubation revealed poikilocytosis and 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 colourless spherical membrane are present. These cell occurrence might be due to the loss of haemoglobin pigment invariably with altered cell permeability.[14]

In case 0.25% Hydro-Alcoholic solution of Turmeric, peripheral smear examination revealed irregular clumping and lysis of RBC was seen.

Smear with 7.5% Hydro-Alcoholic solution of Neem revealed intact surface integrity with RBC and WBC without any lysis and devoid of cytoplasmic vacuolation.

Smear with RBC suspension when treated with isotonic saline revealed intact red blood cell surface. Echinocytes are seen in the peripheral smear. WBC cells were intact without any cytoplasmic vacuolation or disruption.

VII. DISCUSSION

The toxic effects of materials used in endodontic therapy are of particular concern because damage or irritation could cause degeneration of the periapical tissue and delayed wound healing.

Red blood cells were chosen as biological model to evaluate cytotoxic effects as these cells can be easily isolated using least invasive procedure. The red blood cell membranes are semipermeable barriers and the osmotic gradient established on either side of membrane causes the fluid to flow into and out of the cells. The amount of osmotic pressure depends upon the difference between the concentrations of nondiffusible ions on each side of the membrane [15].

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.

The use of 3% 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 viable 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 EL 1985)[8]. From the present study, 3% NaOCl is most cytotoxic at cellular level compared to other irrigants and it should be used with high caution.

Azadirachta indica (Neem) is well known in India as one of the most versatile medicinal plants having a wide spectrum of biological activity. Several studies have proven that neem extract are effective against E. faecalis and C. albicans & as a irrigating solution[16-18].

S Datta Prasad et al. 2014[16] concluded that minimum inhibitory concentration (MIC) of the alcoholic neem extract to E. faecalis & C. albicans were determined as 0.94% & 1.88% respectively. From the present study 7.5% Hydro-Alcoholic solution of Neem is least cytotoxic even at higher concentration.

Several studies have also proven, Curcumin the main yellow bioactive component of turmeric as having a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities [19,20].

The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf-life, low toxicity and lack of microbial resistance [21,22].

The results of this study goes with Pandranki et al 2015. Considering factors such as high antimicrobial efficacy with long term substantivity, least cytotoxicity even on fragile RBC, Morinda tinctoria at higher concentration could be potential alternative to conventional root canal irrigants and might be an adjunctive to the mechanical debridement in endodontic procedures.

Haemoglobin released due to hemolysis with 3% NaOCl is comparatively high when compared to Turmeric, Neem and physiological saline. According to Pashley [8], sodium hypochlorite does not alter the osmotic pressure gradient because of its isotonicity. Hence 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.

VIII. CONCLUSION

Considering factors such as least cytotoxicity even on fragile RBC, Neem and Tumeric could be potential alternative to conventional root canal irrigants and might be an adjunctive to the mechanical debridement in endodontic procedures.

As the present study was conducted on RBC as preliminary trial to evaluate the cytotoxicity, further investigation should be carried out to assess potential of Neem and Tumeric to be biocompatible and effectively disinfect the root canal system.

REFERENCES


