ORIGINAL RESEARCH

Comparative Evaluation of Cytotoxicity of *Neem* Leaf Extract, 2% Chlorhexidine, *Nigella sativa* Extract and 3% Sodium Hypochlorite

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Abstract

To assess and compare the cytotoxicity of different root canal irrigants, namely 25% aqueous *neem* leaf extract (*Azadirachta indica*), 2% chlorhexidine (CHX), 25% aqueous *Nigella sativa* extract (black seed) and 3% sodium hypochlorite (NaOCl). Dulbecco modified Eagle's medium containing glutamine, fetal bovine serum, and antibiotics were used to culture human periodontal ligament cells. After trypsinization, 1×10^5 cell suspension was seeded in 24 well culture plates with 100 µL of culture medium. Then 10 µl of each irrigant was added to the wells, sealed, and incubated in an oven at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Cytotoxicity was assessed at 1, 3, 6, and 24 hours by evaluating the cell viability using the trypan blue assay. A microscope was used for counting viable cells and subjected to statistical analysis. 25% aqueous *Neem* leaf extract was the most cytotoxic irrigant tested, followed by 2% CHX, while 25% aqueous *N. sativa* extract was the least cytotoxic irrigant tested. 25% aqueous *N. sativa* extract and 3% NaOCl solutions were significantly less toxic than 25% aqueous *Neem* leaf extract solution and cytotoxicity of 2% chlorhexidine was significantly more than 25% aqueous *N. sativa* extract and 3% NaOCl at all periods. Considering this encouraging *in vitro* data, herbal extracts could be an alternative root canal irrigant with the most negligible toxicity compared to conventional root canal irrigants, with undesirable effects.

Keywords: Cytotoxicity, Herbal irrigants, *Nigella sativa*.

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Introduction

The success of endodontic therapy depends on thorough cleaning and shaping, with perfect three-dimensional fluid impervious obturation. One of the neglected phases of root canal therapy is proper irrigation and selection of an ideal irrigating solution.[1] Mechanical instrumentation alone cannot provide a thorough and complete debridement of the root canal system, including all of its ramifications and anatomical irregularities. Therefore, irrigating solutions should be used in conjunction with endodontic preparation to eliminate microorganisms from the anatomical intricacies.[2]

Irrigation plays a significant role in endodontic treatment; in both vital and nonvital cases. Blunderbuss canals, perforations, and improper techniques can allow the solution to permeate into surrounding periodontal tissues. Irrigant extrusion occurs even in teeth with fully mature and intact apices.[3] Therefore confinement of the irrigant to the root canal space is of paramount importance. Several irrigating solutions show cytotoxic potential; therefore, when choosing an endodontic irrigant, tissue cytotoxicity should be considered.

Sodium hypochlorite (NaOCl 0.5–5.25%) is a widely used irrigant because it meets the majority of endodontic irrigant requirements better than any other known compound. The ability of NaOCl to dissolve necrotic tissue and organic components of the smear layer is unrivaled.[4,5] However, it has some drawbacks, including high toxicity, an unpleasant taste, corrosive to instruments, inability to remove the inorganic portion of the smear layer, and a decrease in dentin's elastic modulus and flexural strength.[6]

2% Chlorhexidine digluconate (CHX), a biguanide molecule, is another extensively used irrigant. It has extensive antibacterial activity and substantivity, however, it cannot dissolve the organic substrate or necrotic tissue in the root canal system.[4,5] Contact dermatitis, desquamative gingivitis, and tooth discoloration have all been documented as allergic reactions to 2% CHX.[5]

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The global scenario is now changing towards the use of herbal products in dentistry which has been termed as phytodentistry. *Neem* (*Azadirachta indica*) has been regarded as one of the most versatile medicinal herbs in India for over 2000 years, with a wide range of biological activities. *Neem* extract has recently been added to toothpaste and mouthwash. These agents have demonstrated a significant reduction in gingivitis and periodontitis by reducing the plaque levels. It has been reported that *neem* extract has antibacterial action against *Streptococcus mutans*, *Enterococcus fecalis*, and *Candida albicans*. Furthermore, it is readily available and economical.[7]

Nigella sativa (*N. sativa*, NS, Family Ranunculaceae) also called the black seed is a miracle herb with a plenteous historical and religious background, which has many chemical constituents; these phytochemical constituents of *N. sativa* seeds include amino acids, fatty acids, reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glucoside, metarbin, bitter principles, glycosidal saponins, melanthin resembling helleborine, melanthigenin ash, moisture, and Arabic acid.[8] Many pharmacologically active compounds have been isolated from black seeds, but the most reported active constituents are thymoquinone (TQ), dithymoquinone, thymol, and thymohydroquinone. The literature on the therapeutic potential of *N. sativa* in endodontics is scarce, but its miraculous powers are very tempting and need to be explored. Not much study has been performed to check the cytotoxicity of aqueous *N. sativa* extract. Hence this study aims to evaluate the toxic potency of 25% aqueous *N. sativa* extract and compare it with 25% aqueous *Neem* leaf extract, 2% chlorhexidine (CHX) and 3% sodium hypochlorite as root canal irrigant.

Materials and Methods

Preparation of Extracts

Preparation of 25% Aqueous Neem Leaf Extract

One hundred grams of *Neem* leaf powder was bought from IMPCOPS Chennai, India, and wrapped in a muslin fabric that had been soaked in 800 mL of distilled water in a beaker. To generate a 25% concentration of aqueous *neem* extract, the beaker was heated over a low flame until the extract was decreased to 400 mL. After cooling, the extract was filtered using Whatman filter paper no. 1 and kept in an amber bottle.

Preparation of 25% Aqueous N. sativa Extract

N. sativa seed powder was obtained from IMPCOPS Chennai, India. Two hundred fifty grams powder was mixed with 1000 mL distilled water, vortexed until no more color change occurred, and the solution was left at 4°C for 24 hours in a sterile tube. This was centrifuged for 15 minutes at 3000 rpm, and the supernatant (brownish-orange in color) was filtered through Whatman filter paper no. 4 and kept at 4°C in sterile tubes until use.[9]

The study protocol was approved by the ethical committee of Karpaga Vinayaga Institute of Dental Sciences (IRB NO: KIDS/IRB/3–2020/019). The human periodontal ligament fibroblasts used to test the cytotoxicity of irrigating solutions were obtained from periodontal ligament cells of maxillary premolars, which were extracted for orthodontic reasons. Periodontal ligament was carefully removed from the middle part of the root to avoid contamination by the gingiva. The cells were cultured sterile in 75 cm^2 flasks containing 30 mL of Dulbecco modified Eagle's medium (DMEM) with glutamine and 10% inactivated fetal bovine serum (FBS), 50 µg/mL streptomycin and 3 µg/mL amphotericin.

The culture medium was replaced every 2–3 days during the cell culture procedure, and cells were passaged after one week. Cells obtained sufficient confluence for cytotoxicity tests after four passes. The cells were plated into 60 mm culture dishes and maintained at 37°C in a 5% CO_2 and 95% humidity atmosphere until they reached confluence and were withdrawn from the culture medium. After a 5-minute wash with fetal bovine serum (FBS) and ethylenediamine tetraacetic acid (EDTA), the cells were trypsinized by removing this solution and agitating until cells separated.

Treatment with Irrigants

Experimental Groups

- Group 1: 25% Aqueous *neem* leaf extract
- Group 2: 2% Chlorhexidine
- Group 3: 25% Aqueous N. sativa extract
- Group 4: 3% Sodium hypochlorite
- Group 5: Control–Culture medium (Dulbecco modified Eagle's medium)

Centrifugation at 250 rpm for 5 minutes after trypsinization was used to collect specimens and cell groups were distributed in Dulbecco modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS) culture medium. During the fibroblast growth process, the cell count was initially determined on the third day and thereafter daily. Trypsin was administered to float fibroblasts; the solution was then drawn and placed in a test tube. After that, the test tube was centrifuged for 10 minutes at an RPM range of 800–1000. After centrifuging the sediment once more, the finished solution was deposited in the Neubauer chamber. The cell suspension was counted using a Neubauer counting chamber under a microscope (20 µl of suspension contained 1×10^5 cells).

In the 24 well culture plate, the wells were arranged in six rows and four columns, in which five rows were used for depicting five experimental groups and four columns were used for representing four-time periods (1, 3, 6, and 24 hours). 20 µl of suspension was taken from the culture dish with a multichannel micropipette and seeded in 24-well culture plates with 100 µl of culture medium for 48 hours in an oven at 37°C in 5% CO_2 and 95% humidity atmosphere.

A multichannel pipette was used to remove 10 µl of aqueous *neem* leaf extract and was added to the four-time period wells in the Group 1 of the well culture plate. 10 µl of 2% chlorhexidine was added to the four-time period wells in the Group 2 of the well culture plate. 10 µl of 25% aqueous solution of *N. sativa* extract was added to the four-time period wells in the Group 3 of the well culture plate. 10 µl of 3% sodium hypochlorite was added to the four-time period wells in the Group 4 of the well culture plate. No irrigant was added in the control group, which was Group 5. Plates were then sealed and placed in an oven at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Culture plates were always handled in sterile conditions under a Laminar Flow Hood, and sterile multichannel pipette tips were used, which were disposed of on every use to avoid bacterial contamination.

Cytotoxicity Test

Cytotoxicity of irrigating solutions was assessed at 1, 3, 6, and 24 hours after incubation, evaluating the cell viability by using Trypan blue assay.

Trypan blue is a vital stain that is used to color dead tissues or cells blue in a selective manner. It is a type of diazo dye. Color is not added to live cells or tissues with intact cell membranes

because cells are selective about the substances that pass through their membranes. Trypan blue is not absorbed in a healthy cell; nevertheless, it does pass past the membrane in a dead cell. Thus, dead cells appear as a distinct blue color when viewed via a microscope. Since live cells are not stained, this staining approach is sometimes referred to as a dye exclusion method.

At the end of one hour, the 24 well culture plates were taken out of the incubator, the cells were taken from the one hour wells from all the groups respectively with a multichannel micropipette into an Eppendorf tube (1.5 mL) and 0.4% Trypan blue dye was added, mixed gently and allowed to stand for five minutes in room temperature. 10 µl of the stained cells were placed on the Neubauer counting chamber covered with a coverslip and placed on the microscope. The number of viable cells (unstained cells) and the dead cells (stained cells) were counted, and the percentage of viable cells was obtained; this was followed in all groups in 3, 6, and 24 hours, respectively. More number of viable cells at any period implies that the irrigant is less cytotoxic and less number of viable cells indicates that the irrigant is more cytotoxic.

Total number of cells = Total number of viable cells + Total number of dead cells.

Statistical Analysis

Statistical comparison between the groups was made by one-way ANOVA followed by *post hoc* comparison between groups with Bonferroni correction with a significance level of 0.05. Comparison within a time interval of all groups using repeated-measures ANOVA followed by *post hoc* comparison within a time interval of all groups with Bonferroni correction. Data were analyzed using the SPSS 21 package.

Results

Comparison between groups – Table 1 represents the overall comparison of mean values of percentage of cell viability between the groups. Table 2 represents a *post hoc* comparison between two groups at different time intervals.

At 1-hour Time Interval (Fig. 1)

Cytotoxicity of *Neem* leaf extract (*A. indica*) in one hour was significantly more toxic than 25% *N. sativa* extract, 3% NaOCl and control group but not statistically significant to 2% CHX; 2% CHX was significantly more toxic than 25% *N. sativa* extract, 3% NaOCl and control group. There was no statistically significant difference in the cytotoxicity of 25% *N. sativa* extract, 3% NaOCl, and Control groups. However, their cytotoxicity was significantly less than 2% CHX and *Neem* extract. Summarizing the results of all the groups at the end of one hour.

At 3-hour Time Interval (Fig. 2)

The cytotoxicity of *Neem* leaf extract in three hours was statistically not significant when compared to 2% CHX. The cytotoxicity of 2%

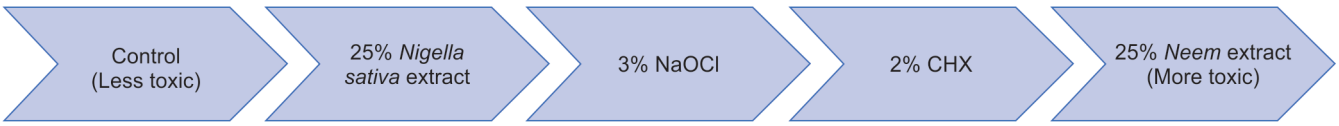

Fig 1: At 1-hour time interval

Table 1: Comparison between groups using one–way ANOVA

					95% confidence interval for mean					
Time interval	*Groups*	*Mean*	*S.D.*	*Std. error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*	*F*	*p-value*
1 hour	1	71.20	2.46	0.63	69.84	72.56	68.00	75.00	62.995	0.001**
	2	72.47	3.60	0.93	70.47	74.46	67.00	79.00		
	3	80.13	1.60	0.41	79.25	81.02	78.00	83.00		
	4	80.00	1.00	0.26	79.45	80.55	78.00	82.00		
	5	80.80	1.86	0.48	79.77	81.83	78.00	84.00		
3 hours	1	74.07	2.22	0.57	72.84	75.30	70.00	78.00	34.689	0.001**
	2	74.73	3.83	0.99	72.61	76.85	69.00	80.00		
	3	81.60	1.45	0.38	80.79	82.41	79.00	84.00		
	4	80.87	1.60	0.41	79.98	81.75	78.00	84.00		
	5	81.40	2.61	0.68	79.95	82.85	76.00	86.00		
6 hours	1	74.73	3.69	0.95	72.69	76.78	69.00	80.00	27.488	0.001**
	2	76.93	3.79	0.98	74.84	79.03	70.00	82.00		
	3	83.33	1.50	0.39	82.50	84.16	80.00	86.00		
	4	82.33	1.80	0.47	81.34	83.33	80.00	86.00		
	5	81.93	2.37	0.61	80.62	83.25	78.00	86.00		
24 hours	1	77.67	5.42	1.40	74.66	80.67	68.00	84.00	14.93	0.001**
	2	79.93	5.23	1.35	77.04	82.83	71.00	88.00		
	3	86.93	1.49	0.38	86.11	87.76	84.00	89.00		
	4	85.07	2.15	0.56	83.87	86.26	81.00	88.00		
	5	86.47	4.85	1.25	83.78	89.15	80.00	96.00		

*p value <0.05 statistically significant
**p value <0.001 highly significant

Table 2: Post hoc comparison between groups with Bonferroni correction

Time interval	*Groups*		*Mean difference*	*p-value*	*Time interval*	*Groups*		*Mean difference*	*p-value*
1 hour	1	2	−1.267	0.133	6 hours	1	2	−2.200*	0.035
	1	3	−8.933*	0.001		1	3	−8.600*	0.001
	1	4	−8.800*	0.001		1	4	−7.600*	0.001
	1	5	−9.600*	0.001		1	5	−7.200*	0.001
	2	3	−7.667*	0.001		2	3	−6.400*	0.001
	2	4	−7.533*	0.001		2	4	−5.400*	0.001
	2	5	−8.333*	0.001		2	5	−5.000*	0.001
	3	4	0.133	0.873		3	4	1	0.331
	3	5	−0.667	0.426		3	5	1.4	0.175
	4	5	−0.8	0.34		4	5	0.4	0.696
3 hours	1	2	−0.667	0.466	24 hours	1	2	−2.267	0.142
	1	3	−7.533*	0.001		1	3	−9.267*	0.001
	1	4	−6.800*	0.001		1	4	−7.400*	0.001
	1	5	−7.333*	0.001		1	5	−8.800*	0.001
	2	3	−6.867*	0.001		2	3	−7.000*	0.001
	2	4	−6.133*	0.001		2	4	−5.133*	0.001
	2	5	−6.667*	0.001		2	5	−6.533*	0
	3	4	0.733	0.423		3	4	1.867	0.225
	3	5	0.2	0.827		3	5	0.467	0.76
	4	5	−0.533	0.56		4	5	−1.4	0.362

*p value <0.05 statistically significant
**p value <0.001 highly significant

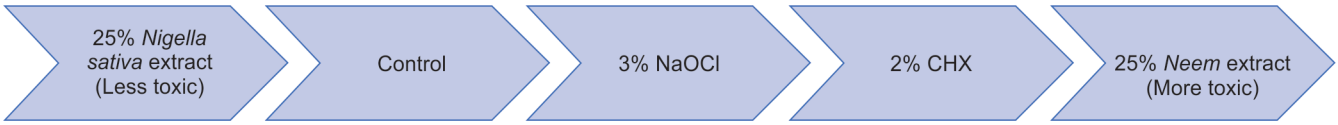

Fig 2: At 3 -hour time interval

Fig 3: At 6 -hour time interval

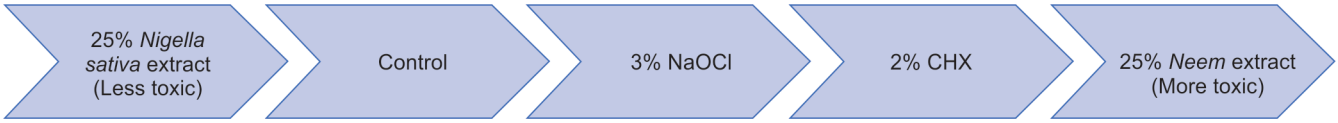

Fig 4: At 24 -hour time interval

CHX was more when compared to 25% *N. sativa* extract, 3% NaOCl, and statistically significant control groups. 25% *N. sativa* extract, 3% NaOCl, and control groups were significantly less toxic than 25% *Neem* leaf extract. Summarizing the results of all the groups at the end of three hours.

At 6-hour Time Interval (Fig. 3)

Cytotoxicity of 25% *Neem* leaf extract in six hours was significantly more than all the other groups; 2% CHX was significantly more toxic than all the other groups except *neem* extract; the cytotoxicity of 25% *N. sativa* extract, 3% NaOCl and control was not statistically significant. However, their cytotoxicity was significantly less than 2% CHX and *Neem* leaf extract. Summarizing the results of all the groups at the end of six hours.

At 24-hour Time Interval (Fig. 4)

Cytotoxicity of 25% *Neem* leaf extract in 24 hours was significantly more toxic than 25% *N. sativa* extract, 3% NaOCl, and control groups. 2% CHX was significantly more toxic than all the other groups except *Neem* leaf extract. Comparing cytotoxicity between the groups of 25% *N. sativa* extract, 3% NaOCl, and control was not statistically significant. However, their cytotoxicity was significantly less than 2% CHX and 25% *Neem* leaf extract; Summarizing the results of all the groups at the end of 24 hours.

Within Groups Comparison

Table 3 represents the overall comparison within a time interval of all groups. Table 4 represents the *post hoc* comparison within

Table 3: Comparison with time interval of all groups using repeated measures ANOVA

Groups	*Type III sum of squares*	*df*	*Mean square*	*F*	*Sig.*
1	316.983	2.015	157.276	9.507	0.001
2	456.45	1.715	266.16	57.936	0.001
3	386.4	2.384	162.051	72.032	0.001
4	221.733	2.189	101.278	40.438	0.001
5	300.983	2.008	149.881	11.411	0.001

*p value <0.05 statistically significant
**p value <0.001 highly significant

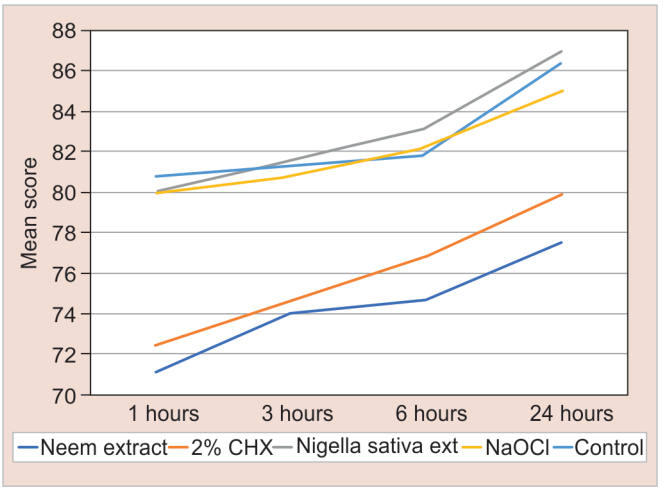

Fig. 5: Mean comparison between and within groups at different time intervals

time intervals of different groups. Figure 5 represents the mean comparison between and within groups at different time intervals. Cytotoxicity of all groups significantly decreased over the period—all materials changing in time-bound.

Discussion

The endodontic irrigating solution should act as an antimicrobial agent but with low periapical tissue toxicity.[10] Endodontic material biocompatibility is determined by several factors, including cytotoxicity, histocompatibility, genotoxicity, mutagenicity, carcinogenicity, and microbial impact. These properties must be explored using a structured battery of various *in vitro* and *in vivo* studies. Any newly discovered material should undergo three distinct levels of testing, ranging from simple to complex test methods, *in vitro* to animal testing, and pre-clinical to clinical testing on people.[11] Since *N. sativa* and *neem* leaf extracts are being evaluated as root canal irrigants, this study was conducted to determine their cytotoxicity in comparison to traditional root canal irrigants such as sodium hypochlorite and chlorhexidine. The cytotoxicity of *neem* leaf extract against human periodontal ligament cells was determined in this study utilizing the Trypan blue assay. The results of the study show that the cytotoxicity of the aqueous *neem* leaf extract was significantly reduced from 1 to 24 hours. 25% of aqueous *neem* leaf extract has shown higher toxicity than 2% chlorhexidine, 25% aqueous *N. sativa* extract, and 3% Sodium hypochlorite. This could be due to the higher concentration of *neem* leaf extract that has been evaluated.

The results of the present study show that cytotoxicity of Group-2 (2% chlorhexidine) is significantly more than Groups 3, 4, and 5 (25% aqueous *N. sativa* extract, 3% sodium hypochlorite, and control, respectively) at the different periods studied. Fernenda Campos et al. evaluated the cytotoxicity of chlorhexidine at various concentrations (0.06%, 0.12%, 0.2%, 1%, and 2%) on odontoblast-like cells and found that 2% was the most toxic. Increased toxicity to cells was observed as the chlorhexidine concentration was increased (from 0.06 to 2%), indicating that chlorhexidine has a dose-dependent toxic effect. Additionally, the toxicity was directly proportional to the duration of exposure.[12] The above finding was also supported by the animal study done by Gisele Faria et al. Their findings showed that chlorhexidine injected in the subplantar space of the hind paw of mice caused severe necrotic changes in the epidermis, dermis, and the subcutaneous tissue at 24 and 48 hours. At the end of 7 and 14 days, the tissue healing was complete.[13]

The use of 3% NaOCl for biomechanical preparation of root canals is a clinically acceptable and highly effective procedure. Previous studies have shown that the antibacterial property of sodium hypochlorite depends on the availability of hypochlorous acid. When the pH reduces, sodium hypochlorite dissociates, and all the available chlorine is in the form of hypochlorous acid.[5] Winter et al. investigated the intracellular effect of hypochlorous acid and demonstrated that it leads to cellular protein aggregation and unfolding, which is responsible for its cytotoxicity.[14] Eduard Hidalgo et al. investigated the bactericidal effectiveness and cytotoxicity of sodium hypochlorite. They explained cytotoxicity

Table 4 Post hoc comparison within time interval of all groups with Bonferronic correction

						95% confidence interval for difference	
Groups	*Time interval A*	*Time interval B*	*Mean difference*	*Std. error*	*p value*	*Lower bound*	*Upper bound*
1	1 hour	3 hours	−2.867*	0.401	0.001	−3.726	−2.007
	1 hour	6 hours	−3.533*	1.272	0.015	−6.261	−0.805
	1 hour	24 hours	−6.467*	1.427	0.001	−9.528	−3.406
	3 hours	6 hours	−0.667	1.149	0.571	−3.131	1.798
	3 hours	24 hours	−3.600*	1.403	0.022	−6.61	−0.59
	6 hours	24 hours	−2.933*	1.336	0.045	−5.799	−0.067
2	1 hour	3 hours	−2.267*	0.284	0.001	−2.876	−1.658
	1 hour	6 hours	−4.467*	0.477	0.001	−5.489	−3.444
	1 hour	24 hours	−7.467*	0.822	0.001	−9.229	−5.705
	3 hours	6 hours	−2.200*	0.416	0.001	−3.093	−1.307
	3 hours	24 hours	−5.200*	0.685	0.001	-6.668	−3.732
	6 hours	24 hours	−3.000*	0.69	0.001	−4.48	−1.52
3	1 hour	3 hours	−1.467*	0.376	0.002	−2.274	−0.66
	1 hour	6 hours	−3.200*	0.428	0.001	−4.117	−2.283
	1 hour	24 hours	−6.800*	0.536	0.001	−7.95	−5.65
	3 hours	6 hours	−1.733*	0.384	0.001	−2.557	−0.91
	3 hours	24 hours	−5.333*	0.583	0.001	−6.583	−4.083
	6 hours	24 hours	−3.600*	0.576	0.001	−4.835	−2.365
4	1 hour	3 hours	−0.867*	0.401	0.048	−1.726	−0.007
	1 hour	6 hours	−2.333*	0.374	0.001	−3.135	−1.532
	1 hour	24 hours	−5.067*	0.483	0.001	−6.102	−4.031
	3 hours	6 hours	−1.467*	0.424	0.004	−2.376	−0.558
	3 hours	24 hours	−4.200*	0.649	0.001	−5.592	−2.808
	6 hours	24 hours	−2.733*	0.573	0.001	−3.962	−1.505
5	1 hour	3 hours	−0.6	0.975	0.548	−2.691	1.491
	1 hour	6 hours	−1.133	0.92	0.238	−3.106	0.84
	1 hour	24 hours	−5.667*	1.275	0.001	−8.401	−2.932
	3 hours	6 hours	−0.533	0.533	0.334	−1.677	0.611
	3 hours	24 hours	−5.067*	1.406	0.003	−8.082	−2.052
	6 hours	24 hours	−4.533*	1.162	0.002	−7.026	−2.04

**p* value <0.05 statistically significant
***p* value <0.001 highly significant

mechanism of sodium hypochlorite lies in the fact that neither bacteria nor human cells can counteract the cytotoxic effect of hypochlorous acid since they lack the enzyme required for its catalytic detoxification.[15] As the antimicrobial effect increases with more available hypochlorous acid, the cytotoxicity also increases.[5] This hypothesis has been proven by Virginie Aubut et al., where they evaluated the cytotoxicity of neutralized 2.5% sodium hypochlorite. This neutralized solution was 10-fold more toxic than its unbuffered counterpart.[14]

Results of our study showed 25% aqueous *N. sativa* extract is significantly less cytotoxic than 2% CHX and 25% aqueous *neem* leaf extract at all tested periods. The seed extract and its constituents appear to have a low level of toxicity. A study done by El Daly 1998 shows five days of intraperitoneal treatment of *N. sativa* seed extract (50 mg/kg) to rats had no significant effect on the activity of numerous enzymes and metabolites indicative of hepatic and renal function.[16] During a 48-hour observation period, oral administration of *N. sativa* seed oil at doses up to 10 ml/kg caused no mortality or overt toxicity in rats and mice.[17] The therapeutic effects of utilizing seeds and thymoquinone appear to be related to their cytoprotective and antioxidant properties, as well as their effect on certain inflammatory mediators.[18]

Limitations

In vitro screening tests such as the cytotoxicity assay are critical for deciphering the fundamental effects of dental materials. However, the methodology has a limitation in that it does not simulate the clinical condition. As a result, it is implausible to extrapolate the *in vitro* findings to the *in vivo* situation. However, a comparable interpretation of the *in vitro* toxicity data provides valuable information about the materials' overall toxic potential.

Conclusion

Within the limitations of the present study, the following conclusions were made, the cytotoxicity of all the irrigants significantly reduced from 1 to 24 hours of the observation period. 25% aqueous *N. sativa* extract and 3% NaOCl solutions were significantly less toxic than 25% aqueous *Neem* leaf extract solution at all periods. Cytotoxicity of 2% chlorhexidine was significantly more than 25%

aqueous *N. sativa* extract and 3% NaOCl. 25% aqueous *neem* extract was not significantly different from 2% chlorhexidine except at 6 hours. The cytotoxicity of the control group was not statistically significant from 25% aqueous *N. sativa* extract and 3% NaOCl.

Clinical Significance

Since irrigating solutions can contact surrounding soft and hard tissues during endodontic treatment, they should have acceptable biocompatibility. Therefore, biocompatibility data of these herbal root canal irrigants concerning cytotoxicity are needed for complete risk assessment before considering it as a choice of irrigation solution.

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