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Alternative Root Canal İrragation Solutions Which Is Non Cytotoxic and High Antibacterial Effectivenes // İn The Case of in Vitro Study Which Is Held in Laboratory

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Abstract

Aim: *Root Canal İrragation solutions and medicine in endodontic treatment is available for to use alternative materials (N acetyl cysteine, boric acid, (chitosan) different concentrations mouse fibroblast cell L929 for to Check the Cytotoxicity and Q. aureus Biofilms for to check antibacterial effectiveness of in vitro aspect evaluation was aimed.*

Equipment Method: *Cell culture test for experiment groups; Chitosan 2048ug/ml- 4ug/ml 10 in different concentration, N Acetyl cysteine (NAC) 50 mg/ml- 0.39 mg/ml between 8 in different concentration, Boric Acid (NA) 64 mg/ml- 0.125 mg/ml between 10 Sodium in different concentration Hypochlorite (NaOCl) 10.5%-5.25 %-2.625% rates 3 different prepared in concentration was created. Antimicrobial test for article concentrations Chitosan 1- 0.002mg/ml, NAC 25- 0.195 mg/ml, Boric acid 32- 0.0625mg/ml aspect was carried out. Prepared microplate At 37ºC 18 hour incubation was released. Study Results group intra- and groups inter- data by comparison analysis was done.*

Findings: *Positive control group the one which... To NaOCl according to all experiment groups more is cytotoxic. Chitosan 128 microgram/ml also first acute toxic the effect of has shown. Q. Aureus on MIC value whereas 0.031 mg/ml is. Antimicrobial dose on the border toxic has been found. N Acetyl Cysteine (NAC) MIC value 1,563 mg/ml while first 24 per hour 25-50 mg/ml in doses toxic It has been found. That is antimicrobial dose on the border toxic It is not has been observed. Boric Acid MIC value 4 While mg/ml This at the rate first 24 per hour cytotoxic not while toxic effect dose and to time connected aspect is increasing. NaOCl all in their concentrations and time in the intervals -best antimicrobial agent found however -most cytotoxic aspect has been observed.*

Conclusion: *Experiment in groups used NAC and Boric Acid antimicrobial dose borderline cytotoxicity in terms of other from groups more Good has been found.*

Keywords: İrrigation, Biofilm ,Cytotoxic ,İnvitro.

Entrance

Endodontics treatment success is to Defencing, root microorganisms from the channel system and so infection is prevented to improve. Coke in the channel bacteria either free floating planktonic only cells aspect either in each other and/or to surfaces adherent microorganism communities the one which... Biofilms aspect It is found (Jiang et al., 2001). Endodontics treatment with bacteria most if it could be eliminated also, the coke channel from the system completely eliminated and more over coke on channel surfaces consisting of A lot various bacterial Biofilms subtract especially It is difficult (Ricucci & Siqueira, 2010). To this According to biofilms to ruin and like this coke channels inside Now bacterial infections eliminate for channel intra- Irrigants And of drugs the use of coke channel increases the success of treatment has been defended (Chávez et al., 2010).

formation to prevent either in consisting of the problem treatment is, most important coke in the channel system observed as a microbial One and that infection eliminate from increasing and invations (Chávez et al., 2010; Lemos et al., 2010).

Today -most chic used irrigation solution sodium hypochlorite (Estrela et al., 2002). Mostly 0.5%; 2.5%; 5.25% concentrations coke channel in preparation in use be This in concentrations effect mechanism necrotic tissue with pus by dissolving antibacterial agent infected to the areas diffusion Providing It is in the form of. However -most big disadvantage highly toxic in high concentrations (Plum et al., 2018; Guida et al., 2006).

Amino acid L Cysteine One derivative the one which... N-acetylcysteine (NAC), in mucus disulfide breaking the bonds and secretions viscosity reducing, thiol including strong One Antioxidant and is a mucolytic agent (El- Feky et al., 2009; Zhao & Liu, 2010). NAC, antibacterial has features non antibiotic One It is a compound. NAC basic effect mechanism, cell female polysaccharides production to reduce, like this mature biofilm to ruin and bacteria to surfaces sticking from reducing consists of (Silver et al., 2013).

Chitosan whereas Kitin by deacetylation in hand said natural One polysaccharide (Rabea, 2010). Antibacterial properties of chitosan and antifungal Features with in dentistry can be used It is defended. Also has been shown in animal experiments (Akkurt & Kitin 2012).

Boric acid herbicidal features showing volatile non- One It is a mineral. Same in time low toxicity owner being together fungicidal effect There is. Gram negative bacterium, -gram positive bacteria and to mushrooms opposite antimicrobial feature can show (Turk et al., 2015; Recai et al., 2013).

In our study; external in medicine endodontic in the field available for use alternative materials (Chitosan, Boric acid, N Acetyl cysteine, (NaOCl) antibacterial their activities and mouse fibroblast origin L929 cell line on their biocompatibility of in vitro aspect review we aimed.

None hypothesis, experimental groups and with the control group There is no difference between two.

Equipment and Method

The study This section two in the section has been realized.

1. Cell The Culture
2. Antimicrobial Effect Cell 1 The Culture

In the experiment to be used Cell Line

In our work used mouse Origin fibroblast L929 cell lineage, ATCC was obtained from the company. L929 cells per week One times routine passages be done by means of, Caucasian University Center In your laboratory replicated. Cells, % 10 fetal cattle serum and 1% antibiotic (penicillin G, streptomycin) including DMEM (4.5 g/L glucose) in the medium 37 At °C, % 5 CO2 And % 95 weather including damp in the incubator, 1 atmosphere pressure under replicated. Sterile culture in their containers produced cells, culture container surface of % 60-80 of them when they cover, experiment was used for.

Chemicals to be solved

Chitosan supplier from the company recruitment was done. 0.5% acetic acid with After it is solved later ph: set to 4.6-4.8. 2048ug/ml- 4ug/ml between ½ sherry dilution by being done 10 different was prepared in concentration.

N Acetyl cysteine (NAC) supplier from the company recruitment was done. Pure This with solved. 50 mg/ml- 0.39 Between mg/ml ½ sherry dilution by being done 8 different was prepared in concentration.

Boric Acid (NA) supplier from the company recruitment was done. Pure This with solved. 64 mg/ml- 0.125 Between mg/ml ½ sherry dilution by being done 10 in different concentration prepared.

Sodium Hypochlorite (NaOCl) supplier from the company recruitment was done. Pure This with solved. 10.5%-5.25%-2.625% rates 3 in different concentration prepared.

The cells to culture Receiving

In experiments used cells, culture their containers % 60-80 at the rate of when they cover, using trypsin/Edta they stick to culture container from the surface removed. Microscope under They were observed to rise to cells Trypsin/Edta effect to obstruct for, equal in quantity serum medium was placed and cell suspension centrifugal in the tube by gathering 1100 at rpm, 2-minute centrifuge was done. Centrifugal post-supernatant was thrown away, the tube at the bottom remainder cells medium on added and only cell suspension to create for pipetting was performed. Hem cytometer by using total cell number of calculated. 96 Well-shaped culture their containers to each of them cell number of calculated, % 100 live only cell from suspension taken 5000 L929 cell planted. Planted cells culture of the container to the surface sticking and proliferation for 37 in °C 24 hours incubate was done.

MTT Preparation

24 hourly incubation finally cells different in concentrations prepared handling chemicals after it was done later 24, 48 And 72. In hours MTT analysis with cell liveliness Effects on was shown. Commercial aspect buy taken MTT 5mg/ml will be in this way pure This with solved. 24, 48 And 72. Hours of finally in the environment medium was removed. In its place 95 ul new medium was added. More 5 mg/ml MTT from the solution 5 ul additional by being in the incubator 4 hour was put on hold. During this time live cells mitochondrial dehydrogenase enzyme with reaction Entering MTT formazan crystals created. More later This Crystal 100 ul DMSO by adding solved and blue- purple in the environment colour formation was observed. 10 min later Results With spectrophotometer 570 Measured by nm' Graphpad prism program help with analysis was done.

Antimicrobial Effect

Stapylococcus aureus MIC And MLC Their values Calculation Bacteria production

Nutrient agar Passaged Q. aureus ATCC 25923 strains At 37ºC 18 hour incubate

Incubation as a result bacterium suspension physiological salty This in MacFarland 0.5 ($1.5 * 10^{8}$ (cfu/ml) separated. 10 ml Muller Hilton broth into 50µl 0.5 MacFarland by adding bacteria MIC, the value of Calculation for was used.

The items preparation

N- acetyl cysteine 50mg weighed. 1 ml distilled This in was resolved. 50 mg/ml mixture was obtained. Boric acid 64mg by weighing 1 ml inside was resolved. 64mg/ml solution in hand was done. Chitosan dissolution for 1ml 0.5% acetic acid (pH: 4.6- 4.8) in 2mg article was resolved.

The items Preparation

N- acetyl cysteine 50mg weighed. 1 ml distilled This in was resolved. 50 mg/ml mixture was obtained. Boric acid 64mg by weighing 1 ml inside was resolved. 64mg/ml solution in hand was done. Chitosan dissolution for 1ml 0.5% acetic acid (pH: 4.6- 4.8) in 2mg article was resolved.

Conducting

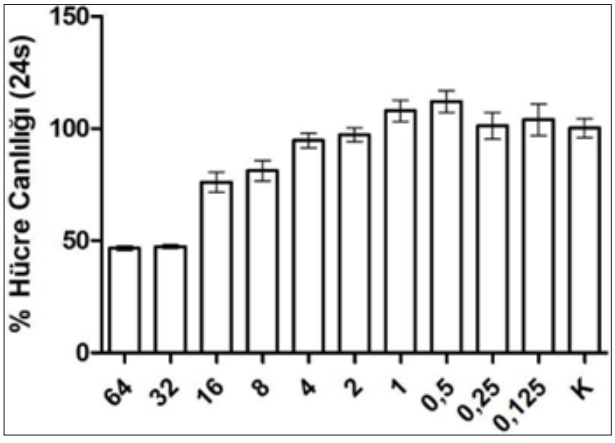
96's microplates first to the well 100µl prepared from substances additional was done. To other wells 50 µl distilled This by being placed first from the well to start as follows 50µl by taking dilution was performed. Prepared dilutions 50 µl prepared bacterium suspension added. Article concentrations bacterium Addition with halfway landed. Thus items final concentrations Chitosan 1- 0.002mg/ml, NAC 25- 0.195 mg/ml, Boric acid 32-0.0625mg/ ml took place. Prepared microplate at 37ºC 18-hour incubation was released.

Incubation finally microplate 600nm wave in the neck spectrophotometer with reproduction by measuring not observed first dilution MIC value aspect was determined. MLC calculate for From MIC value higher from concentrations 10µl by taking nutrient agar October was done. At 37ºC 18-hour incubation result bacterium reproduction It is not -most low concentration as MLC was determined.

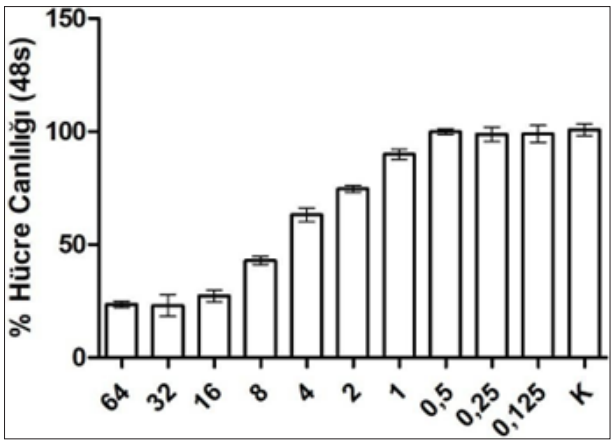
Findings

Cell The Culture Findings

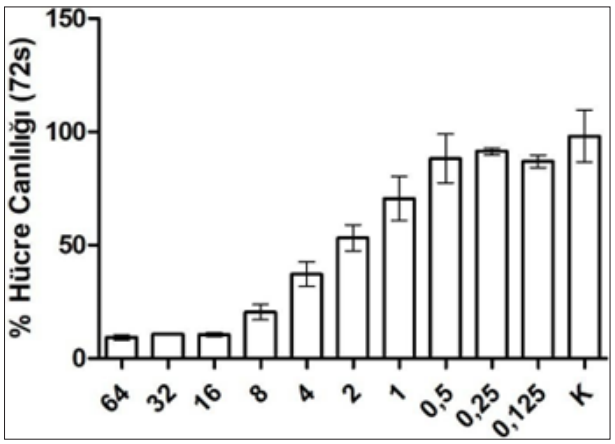
He did it we are in the study first aspect BA's different in doses fibroblast cells on the time connected toxic effect has been researched. 24, 48 And 72. Hour to the results according to BA dose and time connected aspect toxic effect shows.



Shape 1: BA After applied 24 hour later cell liveliness Results (BA: boric acid, doses in mg/ml has been set. Results % aspect calculated.)

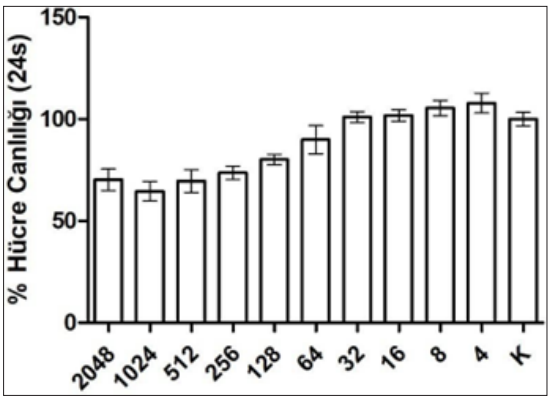


Shape 2: BA After applied 48 hour later cell liveliness Results (BA: boric acid, doses in mg/ml has been set. Results % aspect calculated.)

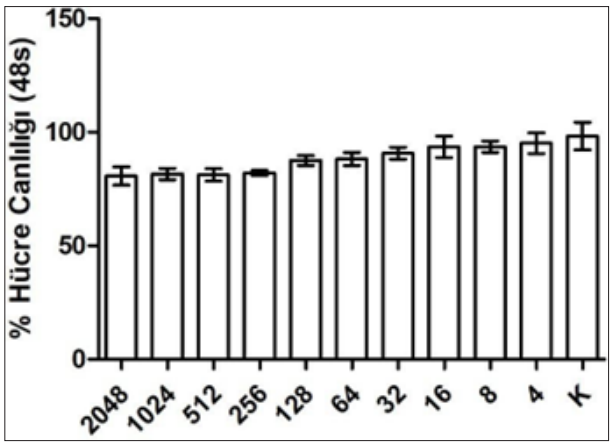


Shape 3: BA After applied 72 hour later cell liveliness Results (BA: boric acid, doses in mg/ml has been set. Results % aspect calculated.)

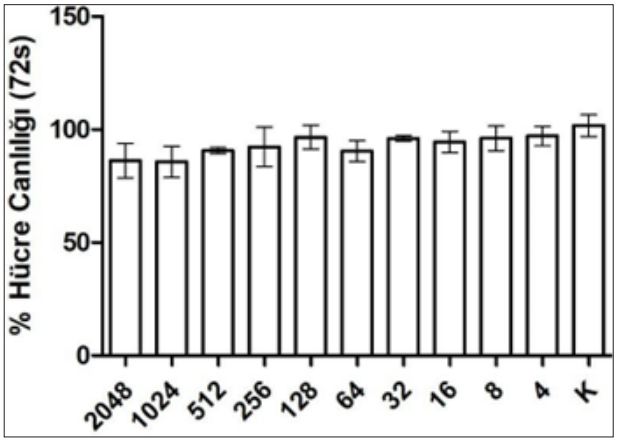
He did it we are in the study second aspect chitosan different in doses fibroblast on the cells to time connected toxic effect has been researched. 24, 48 And 72. Hour to the results According to chitosan acute toxic effect first 24 per hour 128 µg/ml dose and after While being observed This effect dose dependent aspect has not increased, same in doses 48 and in the 72nd hour This effect not observed.



Shape 4: Chitosan After applied 24 hour later cell liveliness Results (doses µg/ml is set to. Results % (calculated as.)

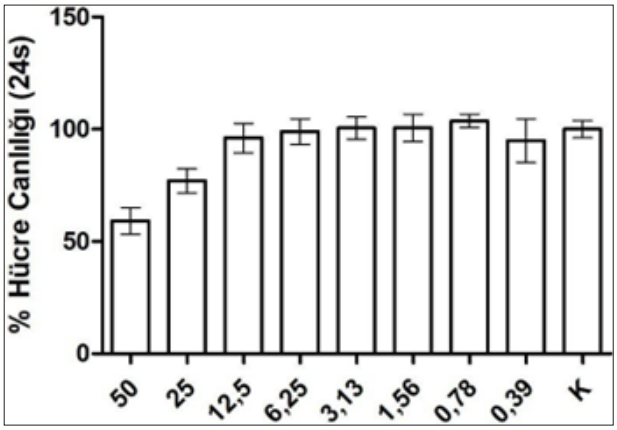


Shape 5: Chitosan After applied 48 hour later cell liveliness Results (doses µg/ml is set to. Results % (calculated as.)

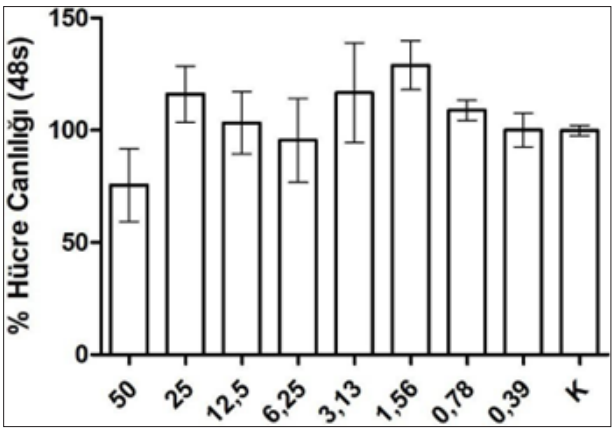


Shape 6: Chitosan After applied 72 hour later cell liveliness Results (doses µg/ml is set to . Results % (calculated as.)

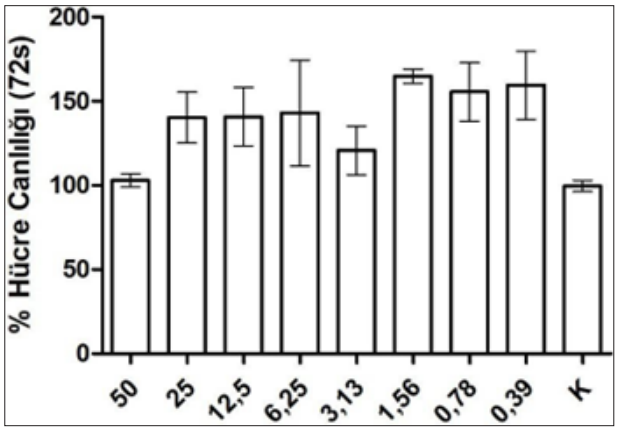
He did it we are in the study third aspect NAC's different in doses fibroblast on the cells to time connected toxic effect has been researched. 24, 48 And 72. Hour to the results According to chitosan acute toxic effect first 24 per hour Only 25 And 50 mg/ml in doses While being observed in the same doses 48 and in the 72nd hour This effect not observed.



Shape 7: NAC After applied 24 hour later cell liveliness Results (NAC: N Acetyl cysteine, doses mg/ml aspect has been set. Results % aspect calculated.)

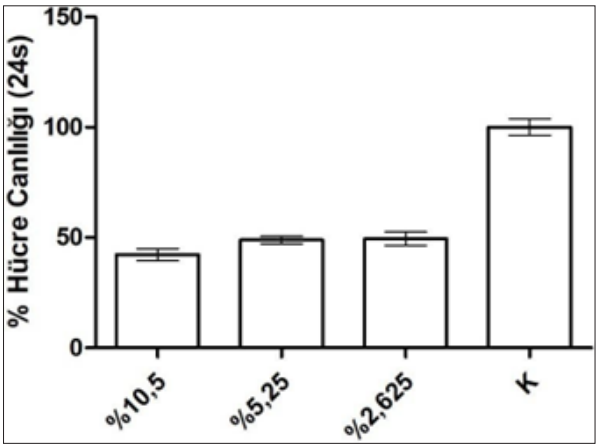


Shape 8: NAC After applied 48 hour later cell liveliness Results (NAC: N Acetyl cysteine, doses mg/ml aspect has been set. Results % aspect calculated.)

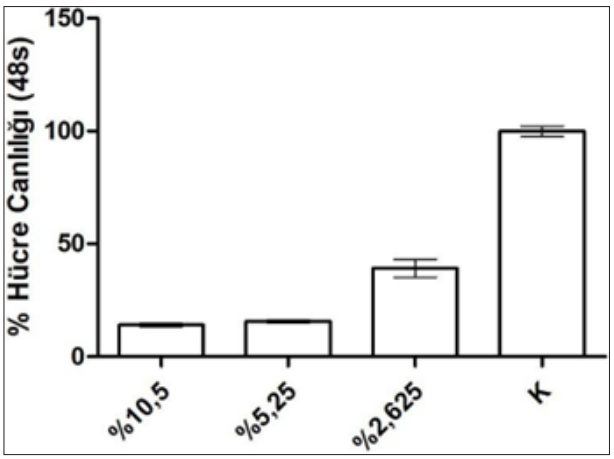


Shape 9: NAC After applied 72 hour later cell liveliness Results (NAC: N Acetyl cysteine, doses mg/ml aspect has been set. Results % aspect calculated.)

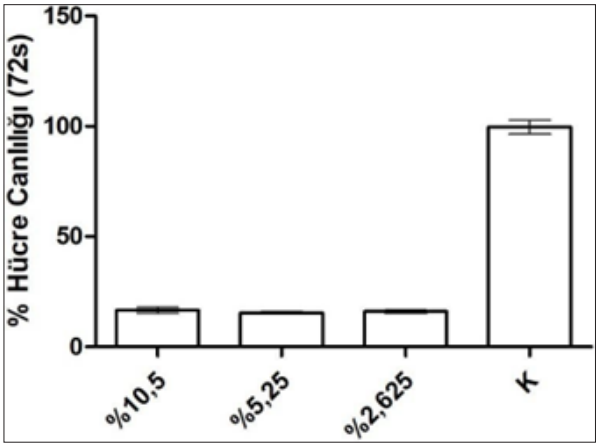
He did it we are in the study last aspect NaOCl different in doses fibroblast on the cells to time connected toxic effect has been researched. 24, 48 And 72. hour to the results according to NaOCl dose and to time connected aspect toxic effect shows.



Shape 10: NaOCl After applied 24 hour later cell liveliness Results (NaOCl: sodium hypochlorite, doses % aspect has been set. Results % aspect calculated.)



Shape 11: NaOCl After applied 48 hour later cell liveliness Results (NaOCl: sodium hypochlorite, doses % aspect has been set. Results % aspect calculated.)



Shape 12: NaOCl After applied 72 hour later cell liveliness Results (NaOCl: sodium hypochlorite, doses % aspect has been set. Results % aspect calculated.)

Antimicrobial Effect Findings

Made study as a result in hand said MIC And MLC below in the table has been stated.

Article	MIC (mg/ml)	MLC(mg/ml)
Chitosan	0.031	1
NAC	1,563	3,125
Boric acid	4	16

Argument

Endodontics in treatment widespread aspect used irrigation solutions though in -Best clinical and biological for effect still the most ideal root channel irrigation solution Searches is ongoing. Moreover, endodontic in treatment used materials Evaluation of their biocompatibility big importance carrying is, biocompatibility any One dental restorative material clinical practically usage for basis from the requirements someone aspect acceptance is stated (Prado et al., 2015; de Gregorio et al., 2010). This in the study N Acetyl Cysteine, Chitosan, Boric acid's different in concentrations mouse fibroblast L929 cells on cytotoxicity And Q. Aureus on antimicrobial activities has been researched. NaOCl positive control group aspect acceptance has been done. None hypothesis was rejected. Experiment in groups both own between them different in concentrations and also control group with between them cytotoxicity and antimicrobial effect in terms of significant difference was observed.

NaOCl in endodontics -most widespread used irrigation solution to be despite high a toxicity has. This for this reason today still To NaOCl alternative Possible irrigation solution search continue is being (de Gregorio et al., 2010).). What we do This in the study alternative Possible other solutions with NaOCl cytotoxicity And Genotoxicity compared.

NaOCl of cytotoxic in studies investigating the effects of Ok and arc. (Ok et al., 2015) 5.25% NaOCl good Bajrami and arc. (Bajrami et al., 2014) to mice Belonging gingival fibroblast in their cells 3% NaOCl, good 2% to CHX according to to solutions according to more cytotoxic has found. Navarro-escobar and arc. of in vitro cell culture with what they did cytotoxicity in his work (Navarro- Escobar et al., 2010). 2.5% NaOCl good more f o u n d it toxic. Ours in our study in mouse fibroblasts in their cells 2.625%, 5.25%, At 10.25% rates NaOCl to time and dose connected aspect cytotoxicity also increase showed and other used experiment in groups from solutions more toxic has been found. What we do result of the study stated with studies compatibility shows.

5.25% NaOCl, 0.2% chitosan, propolis and Humic acid Human gingival fibroblast cells and human osteoblast cells on cytotoxicity of compared to One in the study most toxic materiel NaOCl being present together all solutions APPLICATION duration as its toxicity increases increased has been observed. APPLICATION durations 4- 24 hour is between (Good Luck). In our study in similar in this way NaOCl -most toxic found however Chitosan in the concentrations studied first 24 from the hour later dose and to time connected increase did not show. This difference study in their time and dose from their differences It stems from it could be.

Wu and arc, Q. aureus biofilm to the formation opposite Chitosan hydrogel antibiotic (genipin) added A lot stronger One inhibition was observed and osteogenic activity They stated that he was getting better (Wuet al., 2014).

Sharp And arc, Chitosan nanoparticles (0.3 g/1ml v/v acetic acid), 6% NaOCl, copper added Chitosan nanoparticles antibacterial activity In terms of in t h e i r comparative studies 3 weeks old in incubation Efaecalis on Chitosan nanoparticles with Between NaOCl significant difference did not find, copper additional made Chitosan nanoparticles whereas exhibited significantly better antibacterial activity They stated (Sharp et al., 2021).

A lot study, medical from the perspective important A lot various microorganisms from where is biofilm formation in reducing NAC's its effectiveness has shown (Pei et al., 2018). Coke channel responsible for infections -most important opportunist from pathogens someone the one which... Enterococcus

faecalis on it NAC has antibacterial properties and biofilm no Don't Potential Evaluating in studies writers, NAC's.
TO. faecalis both planktonic both also biofilm to their forms opposite effective is showed (Quah et al., 2012; Stuart et al., 2006).
14 to the day much dentine dust of in existence antimicrobial activity did not decrease. More new a study, NAC's planktonic endodontic to pathogens (Actinomyces naeslundii, Lactobacillus salivarius, Streptococcus mutant and TO. faecalis) opposite strong antibacterial effects that you have and All single type and A lot type bacterium by biofilm formation effective One in a way that prevents reported (Moon et al., 2016). Mature A lot various Biofilms no to be done, 25 mg/mL or higher in concentrations was observed. NAC's both brain heart infusion (BHI) in the medium as well as of in vitro in agar Staphylococcus aureus And Streptococcus pyogenes like wound on pathogens bacteriostatic effects applied has been shown (Yamada, et al., 2011).

NAC in mucus disulfide their ties parts and secretions viscosity lowers (Livingstone et al., 1990). By various bacteria created biofilm formation reducing, antibacterial Features the one which... but antibiotic non- One agent (Schwandt et al., 2004). Quah and arc (2012), pH to 11 raised NAC, E. faecalis planktonic and biofilm to their forms opposite minimum bactericidal concentration.

12.5 mg/ml is reported (Quah et al., 2012). Darrag (2013), different irrigation solutions E. faecalis and Q. Mutans planktonic to their forms and against combined biofilms antimicrobial effect compared and NAC of the solution each two of the species each two to the form opposite demonstrated antimicrobial from the effect because potential One irrigation solution aspect acceptance stated that it could be done (Darrag, 2013). Ours in our study Q. aereus for NAC's MLC value 3.125 mg/ml was found.

Klebsiella pneumoniae, Acinetobacter baumannii, Enterobacter cloaca, Pseudomonas mendocina, Bacillus Cereus and Staphylococcus Warner including to be as follows various gram-positive and gram- negative bacteria growth, 0.4–2 mg/ml NAC in existence completely inhibited is accepted (Olofsson et al., 2003).

Another One in the study (Nunes et al., 2020). NAC's fluconazole sensitive (CaS) And - resistant (Tsar) Candida albicans opposite the effect of evaluated One in the study 50-12.5 mg/ml prepared NAC minimum inhibited impressive concentration (MIC) each two shush for 25 mg/mL has been found. NAC, every two mushroom of its kind viability important to some extent has decreased. Ours In our study, S.Aureus for MIC value more is low.

Selected endodontic to pathogens opposite One intracanal medicine aspect, one antioxidant Mucolytic agent N-acetylcysteine (NAC) antibacterial And Antibiofilm its effectiveness Evaluating i n a study (Moon et al., 2016). Actinomyces naeslundii, Lactobacillus salivary, Streptococcus mutant and Enterococcus faecalis for NAC's minimum inhibited impressive concentrations (MICs), 0.78–1.56 mg/ml MIC values with antibacterial activity showed. NAC, minimum 0.78–3.13 in mg/ml concentrations all only type and A lot type bacteria by biofilm formation significantly hindered has been stated.

Ours In our study in similar aspect Q. aureus on antibacterial activity when investigated MIC value 1,563, MLC value 3.125 mg/ml was. This of in vitro of the study limitations within and other studies when examined, NAC's endodontic against pathogens Good an antibacterial and Antibiofilm to its effectiveness owner is and coke channel in their treatments alternative One channel intra- medicine.

Boric acid in different concentrations (%1, 2, 4, 6) suspensions prepared in one study (Blacklegs et al., 2021). Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis ATCC 29212, Streptococcus mutans ATCC 25175 isolates and blood culture from examples isolated said different resistance has a pattern 20 different microorganisms (Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus epidermidis, Candida albicans, Candida parapsilosis, Streptococcus mitis/oralis) to be as follows total 25 isolate opposite qualitative suspension method with disinfectant effectiveness researched One in the study 4% in concentration 5. from the minute from to all and at 6% concentration 1. from the minute from to all opposite effective is has been found. At 4% concentration 5. from the minute from to all and 6% in concentration 1. from the minute from all opposite effective is has been found. Ours our work Boric Acid Q. Aureus on MIC value 4mg/ml aspect has been found. This finding above stated study with is compatible.

Conclusion

Positive control group the one which... To NaOCl according to all experiment groups more is cytotoxic.

Chitosan 128 microgram/ml also first acute toxic the effect of has shown. Q. Aureus on MIC value whereas 0.031 mg/ml is. Antimicrobial dose on the border toxic has been found.

N Acetyl Cysteine MIC value 1,563 mg/ml while first 24 per hour 25-50 mg/ml in doses was found to be toxic . Well antimicrobial dose on the border toxic It is not has been observed.

Boric Acid MIC value 4 mg/ml while at this rate first in 24 hours not cytotoxic while toxic effect dose and to time connected aspect increase shows.

NaOCl all in their concentrations and time in the intervals -most Good antimicrobial agent found but -most cytotoxic aspect has been observed.

From biocompatibility wood without giving antibacterial materials to develop clinical success Critical for One

importance has. This for the purpose, superior antibacterial activity and biocompatible the one which... for material more LONG to the studies need There is.

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