SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Dentistry

Comparative Cytotoxic Evaluation of Acacia Concinna and Sodium Hypochlorite on Periodontal Ligament Cells - An Invitro Study

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DOI: 10.36347/sasjm.2024.v10i04.010 | **Received:** 27.02.2024 | **Accepted:** 04.04.2024 | **Published:** 22.04.2024

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Abstract **Original Research Article**

Aim & Background: To study the cytotoxic effects of *Acacia concinna* and compare it against Sodium hypochlorite. ***Materials and method:*** Pure methanolic extract of *A.concinna* was prepared using a soxhlet apparatus and concentrated with a rotary evaporator. Human PDL cell fibroblast was seeded in 96-well plates and was divided into 3 groups: Control Group, *A.concinna* extract & 3% Sodium Hypochlorite. All specimens were incubated for 1-hour and 24-hour intervals. After the specified time periods, the test medium was subjected to MTT assay. ***Results:*** Sodium hypochlorite showed the least cell viability at both time intervals. There was no significant difference in cell viability at 24-hour time interval with the sample and control group. ***Conclusion:*** Extract of *A.concinna* showed significantly greater cell viability than NaOCl. Hence this novel irrigant can be an alternative to NaOCl in terms of its biocompatibility ***Clinical Significance:*** This herbal formulation can be used as an alternative to the established hypochlorite for root canal irrigation.

Keywords: Acacia concinna, PDL viability, Cytotoxicity, Hypochlorite, MTA Assay.

INTRODUCTION

Endodontic treatment depends on eradication of micro-organisms and prevention of reinfection from the root canal system which helps to maintain a healthy periodontium [1]. Hence, a combination of chemical irrigants and mechanical preparation of root canal is required for its success [2]. NaOCl though gold standard irrigant, has detrimental effects on peri-apical tissues on extrusion. The sequel of reactions that can be expected include excruciating pain with constant discomfort, diffused swelling, profuse bleeding and ecchymosis [3, 4].

As a shift towards nature, secondary metabolite constituents of herbals have a substantial history with therapeutic importance. Hence an herbal alternative having reduced cytotoxic effect on peri-apical tissues is on search [2]. In that context *Sapindus mukorossi* whose methonalic extract has antibacterial and tissue dissolving properties contributed by saponin [2, 5]. The same triterpene saponin is present in *A.concinna* as well. It is a climber and medicinal plant having significant antibacterial activity. Its fruits rich in saponins act as foaming agent to create lather [2].

Even though various irrigation and irrigant activation techniques have been proposed to avoid inadvertent irrigant extrusion, some limitations in terms of safety or cleaning effectiveness usually remain. Therefore, the irrigating solution should be selected appropriately in these clinical cases and should exhibit a high efficacy in root canal disinfection and debridement, along with the absence of toxicity towards the periodontal tissues. So this study was undertaken to know the cytotoxic effects of *A.concinna* and compare it against Sodium hypochlorite

METHODS

Preparation of Extract of *Acacia concinna*

Organic dried seed pods of *A.concinna* was obtained. Later, it was grounded into fine powder.10g of powdered sample of *A.concinna* was taken and extracted with 150mL methanol using a Soxhlet apparatus at 600ºC for 6hrs.The extract is then concentrated with rotary evaporator to leave a pure sample. The pure extract was stored and refrigerated at 40ºC [6] (Fig. 1 & 2).

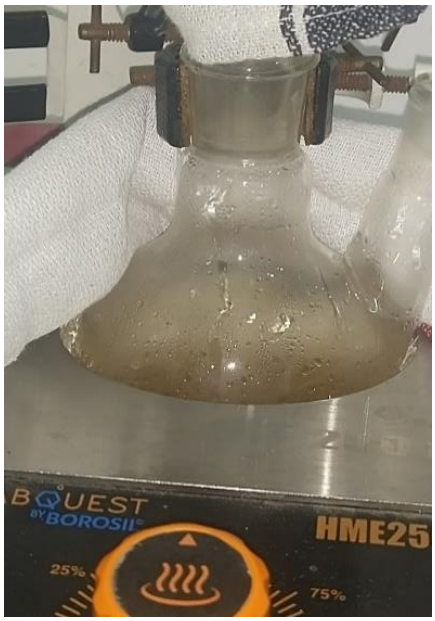

Fig. 1

Fig. 2

Fig.1: Extract getting concentrated in rotary evaporator. Fig.2: Pure Extract of *A.concinna*

Cell Culture Preparation

Human Periodontal ligamental cell lines obtained from the laboratory (WHITE LAB – Material Research Centre, Saveetha Dental College, Chennai, India), were stored in Dulbecco's modified Eagle's medium supplemented with 250 U/mL penicillin, 0.25 mg/mL streptomycin, 0.05 mg/mL gentamycin and 200 U/mL nystatin. The tissue was seeded in 96-well plates and incubated at 37ºC in a 5% CO^2 atmosphere and 95% relative humidity. Afterwards, the cell monolayer was rinsed in phosphate buffered saline (PBS) without calcium and magnesium, dissociated with 0.25% trypsin/EDTA solution, seeded to new 96-well plates at a density of 5×10^3 cells per 100 µL of DMEM and divided in two experiment groups and one control group [7].

Group 1 – Control Group (DMEM)
Group 2 – *A.concinna* extract
Group 3 – Methanol
Group 4 – 3% Sodium Hypochlorite

After the incubation period of 24hr, DMEM was replaced with 100 µL of test medium. The control group will be maintained in DMEM. All specimens were incubated for 1 hr and 24 hr intervals. After the specified time periods, the test medium was subjected to MTT assay. (Fig. 3&4)

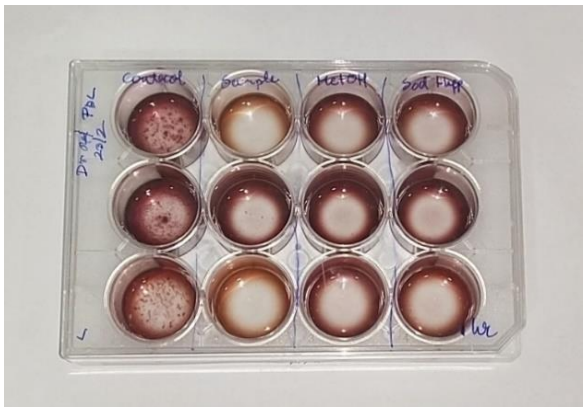

Fig. 3

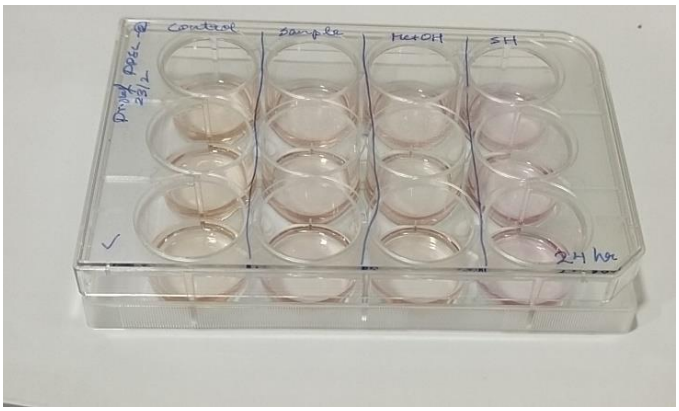

Fig. 4

Fig.3: Samples after incubation at 1 hour interval. Fig.4 : Samples after incubation at 24 hour interval

MTT Assay

The MTT solution was prepared by dissolving 5 mg of 3- (4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide in 1 mL of PBS. After filtering, this solution was diluted 1 to 10 using DMEM; 400 µL of the diluted MTT solution will be added to each well and plates were incubated at 37°C under 5% CO2 and 95% humidity for 4 h. After dissolution of formazan crystals, optical density of the solution will be read at 540-690 nm wavelength using an Elisa Reader. The intensity of colour generated correlated with the percentage of viable cells (Fig. 5) [1].

Fig.5: Samples treated with MTT solution

Statistical Analysis

Triplicate reading was obtained of the test and standard hypochlorite solution. For statistical analysis, Statistical package for the social sciences software (SPSS) was used. Mann Whitney test were used to compare the mean percentage of cell viability at different concentration between two groups. One way Anova test followed by tukey's post hoc test followed by Dunn's post hoc test were used to compare the mean percentage cell viability between different concentrations in tested standard solution.

RESULTS

Table 1 shows cell viability values of all the samples at 1 hour and 24 hour interval. All the control group samples exhibited complete cell viability at 1hr and 24 hr interval. Sodium hypochlorite showed the least cell viability at both time intervals. There was no significant difference in cell viability at the respective time intervals with the sample and methonolic group (Fig. 6).

Table 1: Comparative values of cell viability at 1hr and 24 hr interval

	1 HR	24 HR
CONTROL	100	100
SAMPLE	77.80855	95.87035
METHANOL	73.28533	85.97675
SODIUM HYPOCHLORITE	43.42287	57.35988

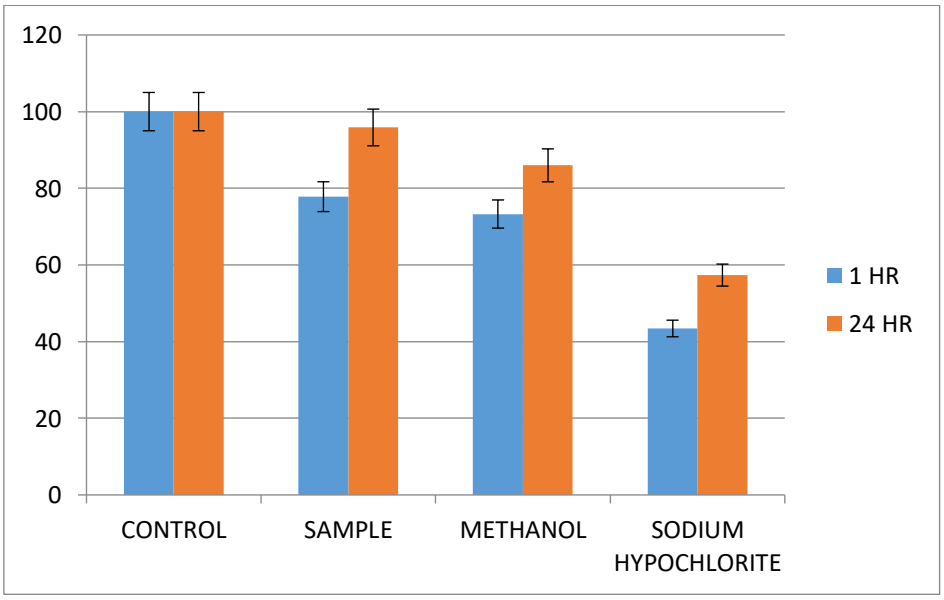

Fig. 6: Bar Graph represents cytotoxicity of the various groups at 1hr and 24 hour interval

DISCUSSION

The present study evaluated the cytotoxic effects of *A.concinna* and compared it against Sodium hypochlorite using MTT Assay. For increasing the chance of success of root canal therapy complete debridement of the root canal system with use of proper irrigants becomes necessary [3]. There always a concern exists on the toxicity of the materials used in endodontic therapy because damage or irritation could cause degeneration of the peri-apical tissue and delayed wound healing. Ideal endodontic irrigating solution should be toxic selectively by acting as an antimicrobial agent but with low peri-radicular tissue toxicity.

MTT is well set up for cytotoxicity analysis of materials, which was previously used for cell viability analysis in the 1980s. This method recognizes the ability of viable cell in changing the water-soluble tetrazolium salts to the insoluble formazan crystals via the activity of mitochondrial dehydrogenase enzymes. Based on the changes in the number of viable cells, cell metabolism and cell morphology this method assesses the cytotoxicity of dental materials. Here, cell damage is underestimated and only cell death, in the apoptotic phase, is detected when cellular metabolism significantly decreases [8].

According to our results, both hypochlorite and test solution induced cytotoxicity in periodontal ligament cells and these impacts were found to be time dependent. Evaluation of cytotoxicity of material in vitro is completely in cellular level. The results acquired by this type of assessment are not adequate for a conclusive clinical evaluation, as in vivo conditions, materials are diluted with body fluids and their concentration tend to decrease and also they are reduced by the action of phagocytes, vascular and lymphatic systems and lastly the inhibitory effect of dentin on irrigants need to be taken [9].

Aqueous NaOCl solution is widely as a root canal irrigant which possesses an excellent tissue dissolving capability, as well as a pronounced antimicrobial efficiency against a broad spectrum of pathogens: Gram-positive and Gram-negative bacteria, fungi, spores and viruses [10]. In addition, NaOCl has the tendency to inactivate or neutralize lipopolysaccharides and has the unique property to disrupt or to remove biofilms [11]. But it is found to have poor smear layer removal [12].

But the cytotoxicity of sodium hypochlorite against the periodontal ligament cells was as low as 43% but increased to 57% at 24 hrs interval due to the high ph which causes oxidative phoporylation and mitochondrial transition pore and finally leading to cell death.

Herbal agents are tried as an substitute for chemical agents like NaOCl due to their low cost,less or no side effects [13]. *A.concinna* is found to have antibacterial, Anti- inflammatory, anti-oxidant, Immuno-modulactory, Anti-cancerogenic potential contributed by Alkaloids, Phenolic compounds, Saponins, Steroids, Tannins and Flavonoids [14].

Saponins can activate T-helper and B- cell which can favor antibacterial activity [15]. The surfactant and emulsion characters of the Saponin reduces the surface tension and provides it with a foaming characteristics [14]. Methanol have been checked for cytotoxic effects in order to understand its influence on addition. No significant difference in cell viability was observed between Group 2 & 3 at 1hour interval. But an increase in cell viability at 24hrs can be observed may be due to initial toxic action of methanol at 1hour interval. But this toxicology has been terminated in Group 2 solution as significant difference in cell viability is appreciated at 24hours interval. From this we can interpret that *A.concinna* is safe to use as a root canal irrigant and if on apical extrusion it can cause least damage to the underlying periodontal cells rather than the Sodium hypochlorite which have severe detrimental effects.

CONCLUSION

Extract of *A.concinna* showed a greater cell viability with periodontal cells in comparison to sodium hypochlorite and both 1hr and 24hours interval. So this extract can be tried as an alternative to sodium hypochlorite. We can conclude from this article stating that this methonolic extract of *A.concinna* is safe to use as an irrigant in root canal space. But further research need to be done to validate its potential in animal and clinical studies to facilitate its use.

Acknowledgements

We acknowledge the support and thank Dr Rajalakshmanan, Saveetha Dental College and White labs CME (Chennai, India) with providing the platform for carrying out with our research.

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