Archives of Veterinary Research Vol. 1 (1): 1-4 (2024)

Open access

Contents lists available at Pensive Academic Publishing

Archives of Veterinary Research

Journal homepage: https://pensive-acadpub.com/

Original research

Comparative evaluation of cytotoxicity of endodontic irrigants in a Wistar albino rat model

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Article history:
Received 11 October 2024
Accepted 12 December 2024
Published 27 December 2024

Keywords:
Root canal irrigants
Cytotoxicity
Grape seed extract
Animal model
Vitis vinifera

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DOI
https://doi.org/10.70964/avr.8

ABSTRACT

Natural products have been used in medicines and are promising sources of novel therapeutic agents. Extensive research worldwide on grape extracts, such as skin and seeds, has exhibited bioactive polyphenolic compounds such as anthocyanins, flavonols and resveratrol. These bioactive compounds demonstrate various biological biological activities such as antioxidant, antimicrobial, anti-carcinogenic and anti-inflammatory. This study evaluated the cytotoxicity of endodontic irrigants in a Wistar albino rat model. Among the tested substances, 10% of grape seed extract proved more biocompatible than EDTA on the seventh day. After 14 days, a significant reduction in the inflammatory process was verified in all experimental groups compared to the control. Still, it was significantly reduced in the Grape Seed Extract group (Group III). The inflammatory reaction observed during the 7 days may be attributed to the superficial necrosis promoted by EDTA.

Introduction

Root canal morphology is complex and contains numerous ramifications and anatomical irregularities. During endodontic therapy, the removal of pulpal and dentinal debris and the elimination of viable microorganisms from the root canal system are of paramount importance [1].

The main objectives of root canal therapy are cleaning, shaping and obturating the root canal system three-dimensionally and preventing re-infection [2].

The success of endodontic treatment is directly influenced by the elimination of microorganisms in infected root canals. Effective antimicrobial agents such as irrigants are necessary for apical periodontitis. Irrigant solutions are very important during root canal preparation because they aid in the cleaning and lubrication of the root canal, have an antimicrobial effect, and dissolve tissue without damaging periapical tissues [3]. The selection of ideal irrigants depends on their action on microorganisms and periapical tissues.

Various concentrations of sodium hypochlorite, chlorhexidine, EDTA, MTAD, and other irrigants have been used during endodontic therapy. The most widely used irrigant is sodium hypochlorite, used in different concentrations varying from 1-6%. Sodium hypochlorite has many drawbacks, such as unpleasant taste, cytotoxicity, and inability to dissolve the smear layer. Also, various studies have shown that sodium hypochlorite, unable to penetrate at greater depths in dentin and long-term exposure of dentin to high concentrations of sodium hypochlorite can have a detrimental effect on dentin elasticity and flexural strength, thereby predisposing the tooth to vertical fracture [4].

Two percent chlorhexidine gluconate (chlorhexidine) has been used as an irrigant and intracanal medicament in endodontics. Chlorhexidine is a bis-guanide that acts by adsorbing onto the cell wall of microorganisms, resulting in leakage of intracellular components. Chlorhexidine has a broad-spectrum antimicrobial activity, targeting both Gram-positive and Gram-negative microbes, and it is biocompatible [5].

The constant increase in antibiotic-resistant strains and side effects of synthetic drugs has encouraged researchers to look for herbal alternatives. Humans have sought to fortify their health and cure various illnesses with herbal remedies for thousands of years. Still, only some have been tried and tested to withstand modern scientific scrutiny. Grape seed extract (GSE) (*Vitis vinifera*) has been well known in India for more than 2000 years as one of the most versatile medicinal plants, having a broad spectrum of biological activity. It is excessively used in ayurvedic, unani and homeopathic medicine. *Vitis vinifera* has been investigated to control many diseases, but its efficacy still needs to be scientifically clarified. Results of various studies show that the extract from *Vitis vinifera* is a powerful inhibiting agent against the increase and the establishment of microorganisms that cause infectious diseases in the oral cavity [6]. The grape seed extract is neither toxic nor has any mutagenic properties. Besides containing long-known bactericidal traits, it has anti-inflammatory, astringent, antiseptic, anti-ulcer, antiviral, antihyperglycemic and immunostimulant properties [7]. Because of the various advantages of grape seed extract, it may be tried as an alternative irrigant to sodium hypochlorite and chlorhexidine since there is literature regarding its irritating potential and cell viability of grape seed extract—the present study aimed to evaluate the cytotoxicity of endodontic irrigants in a Wistar albino rat model.

Materials and methods

Female Wistar albino rats (*Rattus norvegicus*) weighing around 200-250 g were used for this study.

Ethical approval

The study was approved by the Institutional Animal Ethical Committee (IAEC No: CLAR/IAEC/10-2017/005) and was conducted in accordance with their guidelines. Animals were maintained according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Animal grouping

Animals were randomly divided into three groups with n = 6 per group (Table 1).

Table 1. Animal grouping and experimentation

GROUPING	DETAILS	NO. OF ANIMALS
Group I	Control	6
Group II	Bony defect filled with EDTA	6
Group III	Bony defect filled with GSE	6

Surgical procedure

Surgical procedures were performed under sterile conditions in an animal laboratory surgical room. Rats were anaesthetized with ketamine hydrochloride, i.p and xylazine, i.m. at the dosage of 70mg/kg body weight and 10mg/kg body weight respectively. The ventral part of the neck was shaved and aseptically prepared with a solution of Betadine. A 2cm length single median vertical incision was made on the anterior part of the neck, exposing the fascia and muscles underneath. These tissue structures were retracted, and the mandibular bone was exposed. A standardized, round, through-and-through osseous defect, 5 mm in diameter, was created similarly on a single side of the jaw, with a trephine burr mounted on a straight hand-piece driller controlled by the motor regulator and periodically irrigated with saline water. The defect size is consistent with a so-called critical size defect (CSD), implying that the defect does not heal spontaneously during the animal's lifetime. Care was taken during the surgery not to damage the vessels. After filling the defect with the adequate material in respective groups, the tissue flaps are sutured with resorbable sutures (Vicryl 5/0, Ethicon®, Somerville, NJ, USA) and betadine ointment is applied upon the sutured area and then the rats are isolated in separate cages.

The 7th and 14th day were examined for inflammation of the bony defect by sacrificing animals during their respective experimental periods. The mandibular bone containing the defect was dissected and processed for histopathological examination.

Preparation of aqueous grape seed extract

One hundred gms of grape seed powder was obtained commercially, tied in muslin cloth, and soaked in 800 ml of distilled water in a beaker. The beaker was boiled over low flame until the extract was reduced to 400 ml to obtain a 25% concentration of aqueous grape seed extract. After the extract cooled down, it was filtered in Whatman filter paper, and the extract was stored in an amber bottle for further usage.

On one side of the lower jaw, the bone defects were filled with 50 mL of 17% EDTA (Group II) and 10% grape seed extract (Group III), and the bony defect was created and left untreated (Group I).

Histologic preparation

An overdose of pentobarbital euthanized the animals on the 7th and 14th day (n=3 for each day). Lower jaws were excised, and excess tissue was removed. The mandibular part with the defect was fixed in 10% formaldehyde and decalcified in 20% formic acid for 7 days. Afterwards, the samples were embedded in paraffin, and frontal serial sections were cut to a thickness of 7mm. The sections were then processed for Haematoxylin and eosin staining and mounted permanently in DPX.

Article DOI: https://doi.org/10.70964/avr.8

Results

Histologic and histomorphometric evaluation

The histologic evaluation was performed with a light binocular microscope (BX-51; Olympus, Tokyo, Japan) and included a description of the observed tissue response. Five fields around each osseous defect were randomly captured using a 400 magnification to evaluate the number of inflammatory cells in the histological sections. In Group II (EDTA), the lesion site showed infiltration of a few inflammatory cells, whereas Group III (Grape seed extract) showed a marginal level of inflammatory cell infiltration. Nuclei presenting morphologic parameters incompatible with inflammatory infiltrate were manually excluded to avoid interference with cell count. Besides, the software was configured to identify mostly rounded nuclei. The microscope, camera, and computer were calibrated using a standardized procedure for each image capture (Fig. 1 & 2).

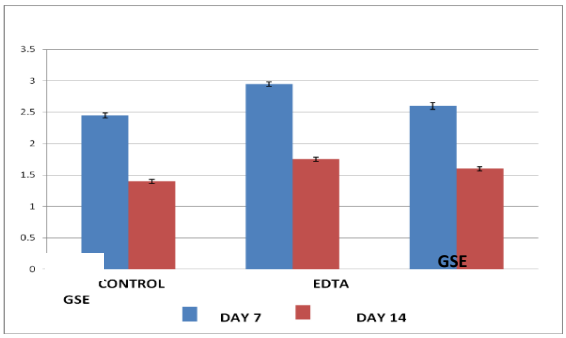

Fig 1. Significant reduction in inflammatory cells was compared between 7th and 14th day

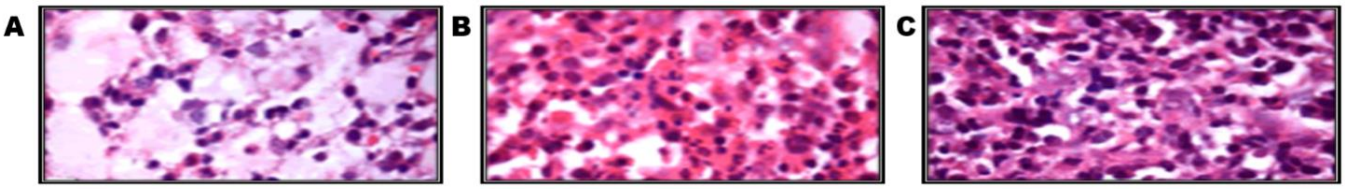

Fig 2. A. Inflammatory cells on the day of placing samples, B. Inflammatory cells on 7th day and C. Inflammatory cells on 14th day

Discussion

Irrigation serves as a physical flush to remove debris and as a bactericidal agent, tissue solvent, and lubricant. Furthermore, some irrigants are effective in eliminating the smear layer. Because of the risk of leakage through the apical foramen, irrigants must be biocompatible and non-irritant to the periapical tissues. Endodontic therapy requires irrigating solutions to eliminate microorganisms, remove debris and neutralize organic compounds [8]. In endodontic treatment, sodium hypochlorite (NaOCl) and chlorhexidine (CHX) have been used for root canal irrigation. Sodium hypochlorite has been widely recommended as an irrigation solution to aid in the chemomechanical debridement of the root canal system because of its dissolving action on pulp tissue and antimicrobial properties [9].

Recent findings about root canal medicaments and irrigating solutions showed that some irrigant solutions may be potentially toxic and may cause damage to the surrounding tissue [10]. The endodontic irrigating solution should be selectively toxic and act as an antimicrobial agent with low periapical tissue toxicity [11]. This study has been undertaken to evaluate the cytotoxicity of Grape seed extract and compare it to standard root canal irrigants like sodium hypochlorite and EDTA.

Morphological and Quantitative Analysis

After 7 days of treatment, the region of bony defect showed infiltration of numerous inflammatory cells, mainly lymphocytes and macrophages. A significant decrease in the number of inflammatory cells and multinucleated giant cells was verified from 7 to 14 days in all groups. The specimen consists of a cellular fibrous stroma surfaced by a stratified squamous epithelium. The stroma is infiltrated by acute and chronic inflammatory cells displaying lymphocytes, plasma cells, polymorphonuclear neutrophils, and macrophages. The number of inflammatory cells was quantified and graded based on the severity of inflammation. The inflammatory cells were much reduced in the GSE group than in the EDTA group compared to the control group.

We investigated the anti-inflammatory effects of grape seed extract on periapical tissues by histomorphometric and histological means. Our histomorphometric findings showed inflammation and destruction in all the groups in different severities using inflammatory cell infiltration, connective tissue attachment loss, and osteoclastic

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activity. The grape Seed Extract improved connective tissue levels and was associated with lower inflammation than the control group (Group I). These findings may be supported by the results of some studies in which GSE decreased reactive oxygen and nitrogen species, inhibited myeloperoxidase and lysosomal enzyme activities in experimental periodontitis, downregulated matrix metalloproteinases in inflamed periodontal tissues [12], and inhibited osteoclast differentiation and reduced osteoclast activity in mice when applied as 100 mg/kg for 18 days 23. Although the doses, methods, and periods of GSE application are dissimilar in these studies, all their findings show positive effects of GSE on bone formation and healing.

There is a growing interest in herbal remedies as adjunctive anti-inflammatory agents in preventing periodontal disease, particularly for individuals prone to disease [13]. This study evaluated the interaction between grape seed extract and EDTA in the periapical region. Histopathological findings showed improvements in the inhibition of inflammation and destruction following GSE.

Conclusion

According to our methodology, 10% of grape seed extract was more biocompatible among the tested substances than EDTA on the seventh day. After 14 days, a significant reduction in the inflammatory process was verified in all experimental groups compared to the control. Still, it was significantly reduced in the grape seed extract group (Group III). The inflammatory reaction observed during the 7 days may be attributed to the superficial necrosis promoted by EDTA.

Disclosure statement

The author reported no potential conflict of interest.

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Article citation

ArunKumar, Krishnaprakash G, Nagaraj NJ, Karunajothi E, Mavishna MV and Keerthi R (2024). Comparative evaluation of cytotoxicity of endodontic irrigants in a Wistar albino rat model. *Archives of Veterinary Research*, 1(1), 1-4.

Article DOI: https://doi.org/10.70964/avr.8

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