J Oral Res
oral and craniofacial sciences

Conference paper

The effects of luteolin nanoparticles on the healing of extracted tooth socket in rabbits.

Maha T. Al-Saffar.[1]
Amer A. Taqa.[1]

Affiliations: [1]Department of Dental Basic Science, College of Dentistry, Mosul University, Iraq.

Corresponding author: Maha T. Al-Saffar. Dept of Dental Basic Science, College of Dentistry, Mosul University, Iraq. E-mail: Mahatala72@yahoo.com

Abstract: Objective: To evaluate the effects of luteolin nanoparticles on the process tooth socket healing in rabbits. Design: This study comprised five rabbits randomly assigned to control animal and experimental animals. Immediately after the extraction of an upper maxillary incisor, the alveolar sockets of experimental animals were treated with topical luteolin while alveolar sockets of the control group remained without treatment. The animals were sacrificed by decapitation with deep anesthesia seven days post tooth extraction. The tooth sockets were sectioned and stained with hematoxylin and eosin stains. Results: Histological evaluation revealed that luteolin treatment induced earlier healing of extracted tooth sockets. Conclusion: These findings suggest that luteolin accelerates the healing process in tooth sockets of rabbits.

Keywords: Luteolin; wound healing; tooth extraction; tooth socket; models, animal.

INTRODUCTION.

Wound healing, as a normal biological process in the human body, is achieved through highly programmed and dynamic phases. For successfully healing, all stages must occur in the correct sequence. Observation of post-extraction healing after surgery is an important issue in dentistry; recently there are many studies that have been carried out in order to evaluate the histological changes in uncomplicated oral wound healing.[1]

Healing occurs in three stages, an inflammatory phase, a proliferative phase, and a maturation phase. An early clot forms as a coagulum of white and red blood cells in the same ratio as is found in the circulating blood; the clotting is supported by sheets of fibrin. Healthy granulation tissue replaces the clot over a 4-day to 5-day period.[2]

This tissue is composed of remaining red blood cells, a specialized influx of white blood cells, endothelial cell cord-like structures and reticuloendothelial cells; associated with capillaries, connective tissue usually replaces granulation tissue within 14-15 days. The new preosseous tissue is categorized by the arrival of young rod-shaped fibroblasts, collagen fibers, metachromatic ground substances, and the manifestation of alkaline phosphatase, bone creation begins on the seventh day with the development of a fibrillar, poorly calcified osteoid at a more profound area and at the periphery of the socket, termination of epithelium begins as renewal on

Conflict of interests: No conflict of interests.

Ethics approval: Approved by ethics committee of University of Mosul under license number2 at 13-06-2018.

Funding: The research is self-funded.

Authors' contributions: Maha Talal Al-Saffar: the experimental work on animals. Amer A. Taqa: Preparation of the material and chemical analysis of the results.

Acknowledgements: None.

Cite as:
Al-Saffar MT & Taqa AA.
The effects of luteolin nanoparticles on the healing of extracted tooth socket in rabbits.
J Oral Res Special Issue. 2019;S1:15-18.
doi:10.17126/joralres.2019.084

the fourth day, with a complete union in closure of the socket after 24–35 days. Mast cell migration into the epithelium from the primary connective tissue begins during the first week and is essentially completed after 7 weeks.[3] Luteolin (3,4,5,7-tetrahydroxy flavone) is one of the most popular flavones, which is usually found in a few edible plants, and in traditional medicine due to its properties.

Luteolin has antioxidant potential and bacteriostatic properties, and it is also antinociceptive, antiangiogenic, anti-inflammatory, antimutagenic, anticarcinogenic, and is effective in the treatment of different diseases including peptic ulcers. Also there are multiple pieces of evidence on the wound healing effect of luteolin on diabetic rats.[4] The aims of this study were to estimate the effect of topical application of luteolin nanoparticle in the acceleration of extracted tooth socket healing in healthy rabbits.

MATERIALS AND METHODS.

Five healthy local rabbits were used in this study. The weight of the animals ranged 1.5–2.5kg and their age was between 10–12 months. The animals were kept in a room with good conditions, with an alternate cycle of light/dark photoperiod at 25-30°C with 50–65% humidity. All animals were served the same diet which consisted of carrots, dry bread, lettuce, radishes and wheat. Tap water was also provided *ad libitum*.

The study was carried out according to the guidelines of international medical research for the use of animals. All measurements were taken to prevent animal cruelty under the approval of the ethical committee of the Mosul University, Iraq.

Experimental design

The dose of anesthesia was calculated according to animal weight. The animals were anesthetized by using general anesthesia xylazine (Holland, Castenrary, Interchemra) 5mg/kg intramuscularly in combination with ketamine 35mg/kg (India Pharmaceutical Ltd.) The anesthesia was carried out before extraction and before each luteolin application. The right first upper central incisor was extracted from all rabbits after 10 min of anesthetic administration.

The tooth usually luxates by applied a straight force, and when the tooth loosens it is then gently rotated in the socket and pressed towered apical germinal tissue for destruction, as has been usually done to prevent re-growth. Then the tooth was extracted in lingual movements. Luteolin (Yanhuang Industrial Park, Guanxian, Liaocheng, Shandong, China) was applied immediately in the socket after extraction until the socket was completely filled and every 24hr for the next 7 days. (Four rabbits were treated while the tooth socket of the fifth rabbit remained empty as a control).

All five rabbits were sacrificed at 7 days. Block section of the maxilla that contained the tooth socket with the boundary of adjacent bone was removed, and the specimens were fixated in 10% formalin, and then were embedded in paraffin; histological sections were taken coronally of 5 μm thickness. Hematoxylin and eosin (H&E) were used to stain the sections for routine examination.[5]

Histological analysis

A blind examiner performed the following histological analysis with a light microscope. Slides were studied at 40x, 100x, 200x magnification to check epithelial proliferation, granuloma tissue formation and organization, newly formed capillaries, and the sections were qualitatively assessed for fibroblast proliferation, collagen maturation, angiogenesis, and epithelialization.

RESULTS.

Histological studies of both treated and control extracted sockets after seven days. (Figure 1)

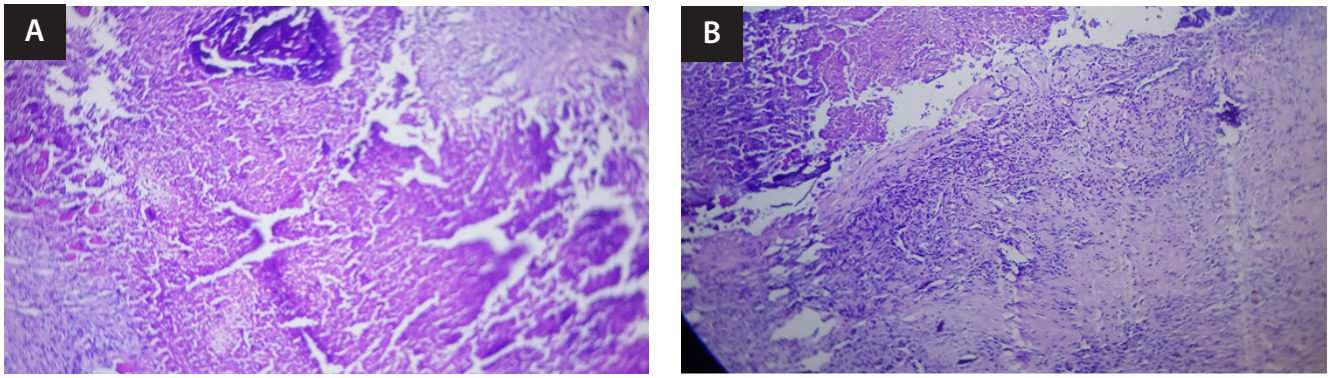
It can be observed that in the control socket the cavity is filled with necrotic material with no signs of healing, while in the treated socket the amount of necrotic material present was much lower and there is presence of granulation tissue at the deeper areas of the socket cavity, with hemorrhagic areas and also the presence of inflammatory cells.

There was no trabecular bone in either the control or any of the treated sockets at 7 days. (Figure 1)

Photomicrographs of a histological section of the extracted treatment animal within 7 days of tooth extraction. H & E stain x 40 and 100x.

ISSN Online 0719-2479 - www.joralres.com © 2019

Figure 1. Photomicrographs of H&E stained of tooth socket histological sections at 7 days post-extraction (x 40 and 100x magnification).



A: Socket treated with luteolin. **B:** Socket of control animal.

DISCUSSION.

This is the first study attempt to assess the effects of luteolin nanoparticles in the acceleration of extracted tooth socket healing in rabbits. The rabbit model was used in this study as it is easier to manipulate in this type of interventions compared with other animals such as rats or mice. These animals also have primitive bone structures but do not have haversian systems. However, the healing process occurs rapidly in these animals compared to humans, which is an additional advantage for further studying comparable technologies, but have disadvantages as results cannot be directly extrapolated to humans. Other studies have estimates the effects of different materials like platelet-rich fibrin, flavonoid-rich fractions, bisphosphonate and parathyroid hormones, human bone grafts, and honey on the healing of extracted tooth socket.[6-7]

Luteolin (3,4,5,7-tetrahydroxy flavone) is an important flavone, which is usually present in several plants. Chemically, it has a C6-C3-C6 structure, which usually has two benzene rings and one oxygen-containing ring with a C2-C3 carbon double bond. Structurally, the presence of hydroxyl moieties at carbons 5, 7, 3 and 4 positions of the luteolin structure and the presence of the 2 - 3 double bond usually have different pharmacological benefits or effects.[4]

Luteolin, usually present as a glycosylated form, has antioxidant, anticancer, anti-inflammatory, and neuroprotective effects, therefore it choosen to be assessed in the present work. The results of this study demonstrate that luteolin enhances healing of extracted tooth socket and reduces inflammation, in agreement with other studies which demonstrated that luteolin inhibits the inflammation process caused by keratinocytes and in a mouse psoriatic experimental model. Inflammation is a physiological event that occurs in response to tissue harm caused by bacterial infection or due to chemical irritation, or wounds.[8]

Inflammation is normally considered a part of the wound healing process that combats harmful microorganisms. This blood derived inflammation fluid usually contains a high amount of fibrinogen that coagulates at the wound site and in the adjacent tissues. The coagulated fibrin will later convert into a dense, attached scar, as such excessive swelling must not be permitted.[9]

Many different mechanisms appear to have a role in the anti-inflammatory activity of luteolin. Luteolin has a role in preventing NF-kappa B activity at micromole levels that enhance the expression of pro-inflammatory chemokines and cytokines. Some researchers have demonstrated that luteolin can inhibit COX-2, LOX, and iNOS, this may explain its anti-inflammatory effects.

Luteolin also has a very effective role in inhibiting enzymes responsible for the synthesis of leukotriene B4 and thromboxane B2, in addition to hydrogen peroxide scavenging activity. Luteolin has previously exhibited a very strong effect in preventing the activity of both leukotriene

and thromboxane synthesis. The same study also showed that the effect of luteolin and its related glycosides against hydrogen peroxide and arachidonic acid synthesis scavenging are dependent on their molecular structures. The appearance of the ortho-dihydroxy ring in the B-ring and OH substitution pattern at the C-5 position of the A-ring could significantly explain the anti-inflammatory and antioxidant activities.[10] The valuable effects on the healing of socket of the luteolin nanoparticles employed in this study can be inferred to facilitate the process of healing of different clinical conditions, such as alveolar osteitis treatment, which occurs after tooth extraction.

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