Abstract. – OBJECTIVE: Our aim in this study was to investigate the effect of Gallic acid (GA) on gingival tissue injury.

MATERIALS AND METHODS: Twenty rats were categorized into two groups. In the burn group, an excisional wound area was created by removing a 4 mm diameter flap from the left molar region in the mucoperiosteal region of the gingiva. In the Burn+gallic acid group, 1.2 mg/ml GA was administered as irrigation for 1 week. Animals were sacrificed under anesthesia at the end of experiment. Malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) levels were measured. Hematoxylin Eosin, fibroblast growth factor (FGF) and epidermal growth factor (EGF) immunostaining were applied to tissues.

RESULTS: MDA and MPO levels increased, and GSH, epithelization, FGF and EGF expression levels were decreased. Gallic acid treatment improved these scores. Degenerated gingival epithelium, disintegrity in epithelial and connective tissue fibers, edema and inflammatory cells were observed in the burn group. Gallic acid treatment after burn improved the pathologies. After burn injury, FGF and EGF activity was increased in Gallic acid-treated groups.

CONCLUSIONS: We suggest that GA has the potential for better healing outcomes in oral wounds. GA seems to have promising therapeutic efficacy in enhancing oral wound healing.

Key Words: Gallic acid, Gingiva, EGF, FGF, Antioxidant.

Introduction

Current therapies for oral wound healing lack effective treatment outcomes in oral wound management and tissue regeneration. The lack of successful therapy for oral mucosal wounds has forced clinicians to find out alternative treatments and potential therapies to facilitate intraoral healing. Following injury, the oral mucosa undergoes several biological healing processes for homeostasis. While wound healing is known and treated in cutaneous wounds, there is limited literature in intraoral healing, complicating clinical treatment alternatives. In the case of impaired wound healing, the oral cavity is sensitive to problems arising from postoperative complications, trauma related injury, prolonged inflammation1,2. After wound injury, macrophages secrete cytokines including interleukin-1, interleukin-6, fibroblast growth factor (FGF) and epidermal growth factor (EGF)3. Remodeling of tissue occurs and that provides pro-regenerative growth factors like FGF, EGF, and VEGF4. Fibroblasts migrate to the provisional matrix and are integral for extracellular matrix (ECM) remodeling; these cells lay down matrix proteins, including collagen and fibronectin, to provide structural integrity of the healing tissue5. EGF and FGF are growth hormone involved in cell proliferation, cell growth and cell differentiation during wound injury6,7. Gallic acid (GA), (3,4,5-trihydroxybenzoic acid) is a polyhydroxy phenolic compound and its structurally related compounds are widely found in fruits and plants. GA esters have a diverse usage in industry, as antioxidants in food, in cosmetics and in the pharmaceutical industry. GA is a source material for inks, paints and color developers. Research8 showed that these compounds are potential therapeutics with anti-cancer and antimicrobial properties. GA was found to possess anti-inflammatory activity. Scavenging of superoxide anions, inhibition of myeloperoxidase release and activity and interference with the assembly of active nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase may be the reason for the inhibition of inflammatory process by gallic acid9.

In this study we aimed to investigate the role of gallic acid against tissue injury induced by gingival burn injury in rats by biochemical, histopathological and immunohistochemical methods.
Materials and Methods

All experimental protocol was approved by the Local Ethical Committee of Animal Experiments of Dicle University, Turkey. 12-week-old Sprague Dawley rats were fed in stainless steel cages at 22±2°C with normal diet and tap water for 12 hours in light and 12 hours in darkness without any restriction.

Experimental Design

Using 90 mg/kg ketamine hydrochloride and 8 mg/kg xylazine (intramuscular) under general anesthesia, sterilization with Povidone iodine solution was provided, and an excisional wound area was created by removing a 4 mm diameter in the gingiva located in the left molar region. Irrigation agents was started to be applied in the form of 1 cc irrigation solution after injury is created and was given as 30-second applications once a day at the same time every day. Buprenorphine was used as a post-traumatic analgesic. A single dose of 0.01 mg/kg subcutaneous was administered for postoperative analgesia. Gallic acid (catalog No.: 147915) was imported from Merck (Darmstadt, Germany). Irrigation solutions was prepared as 1.2 mg/ml in 1 cc.

Burn group: The animals in this group were injured in the gingiva, and the animals in this group were sacrificed by intracardiac blood collection after 1 week.

Burn+GA group: Wounds was created in the gingiva of the animals in this group, and 1.2 mg/ml GA was administered as irrigation for 1 week. Animals were sacrificed by intracardiac blood draw under anesthesia at the end of the experiment.

Biochemical Analyses

Blood samples were collected in tubes with a gel separator and centrifuged for 5 minutes at 1,550 g. The supernatant plasma was removed and placed in polypropylene plastic tubes. The tubes were properly labeled with the appropriate sample name and type. Samples were taken and stored at -80°C for the determination of the Malondialdehyde (MDA), Glutathione (GSH) and Myeloperoxidase (MPO). MDA levels were determined using the double heating method of Draper and Hadley10. MDA values were expressed as nanomoles per gram (nmol/g) of wet tissue. The GSH activity was measured by the method of Paglia and Valentine11. Data were expressed as U/g protein. Myeloperoxidase (MPO) activity in tissues was measured by a procedure similar to that described by Hillegas et al12. MPO is expressed as U/g tissue.

Histopathological Analysis

Gingival sections were obtained for histopathological analysis and were fixed in 10% buffered formalin, dehydrated in ethanol (50% to 100%), purified in xylene, and embedded in paraffin. Sections (4-5 mm thick) were cut and stained with hematoxylin and eosin (H-E). The sections were studied to assess the pathological changes in the gingiva tissue13,14.

Immunohistochemical Analysis

Formaldehyde-fixed gingival tissue was embedded in paraffin wax for further immunohistochemical examination. Gingival sections were deparaffinized in xylene and passed through descending alcohol series. The antigen retrieval process was performed in citrate buffer solution (pH 6.0) for 15 minutes in a microwave oven at 700 W. Sections were allowed to cool at room temperature for 30 minutes and washed twice in phosphate buffered saline (PBS) for 5 minutes. Endogenous peroxidase blockage was performed in a 3% hydrogen peroxide solution for 7 minutes. The washed samples were incubated in Ultra V block (catalog No. TA-015UB, ThermoFisher, Waltham, MA, USA) for 8 minutes. Blocking solution was removed from the sections and allowed to incubate overnight at +4°C with primary antibodies EGF, (catalog No. ab9695, Abcam, Waltham, MA, USA) and FGF (catalog No. ab92337, Abcam, Waltham, MA, USA). After washing the sections in PBS, secondary antibody (TP-015-BN, ThermoFisher, Waltham, MA, USA) was applied for 20 min. The sections were washed in PBS for 2x5 min and then exposed to streptavidin-peroxidase (TS-015-HR, ThermoFisher, Waltham, MA, USA) chromogen. Counterstaining with hematoxylin was applied and after washing, the preparations were mounted. Sections were examined under a light microscope (Zeiss Imager A2, Oberkochen, Germany)15.

Statistical Analysis

Statistical analysis was performed by the SPSS 25.0 software (IBM Corp., Armonk, NY, USA). Data distribution was analyzed with Shapiro-Wilk test. The data were recorded as median (minimum-maximum) with mean rank value. Binary group comparisons were evaluated with Mann-Whitney U and p<0.05 was accepted as the significance level.
Results

Statistical analysis of biochemical (MDA, MPO and GSH) and histological parameters (epithelization, inflammation, leukocyte infiltration, FGF and EGF expression) were shown in Table I. Compared to control group, GSH, epithelization, FGF and EGF expression were statistically increased. Inflammation and leukocyte infiltration were decreased, but it was not significant. MDA and MPO level were significantly lower in burn+gallic acid group than in burn group.

Graphical illustration of Table I and histological staining were shown in Figure 1. We can say that gallic acid induces epithelial structuring of papillary structures extending from the connective tissue to the epithelium, although there are partially degenerative changes in the gingival epithelium.

Discussion

Unsuccessful healing can lead to scar formation and impaired growth of the palate and dento-maxillary complex\(^2\). Therefore, to reduce impaired healing following oral surgery, there are ongoing research projects to improve this situation. Alternative strategies are being investigated in order to promote oral wound healing and tissue regeneration in conjunction with surgical intervention. Different new therapeutics were investigated\(^{16,17}\) as a treatment option with the use of synthetic polymers, biological grafts, gel-like ointments, hybrid scaffolds, and drugs, cells, tissue, or growth factors to enhance oral wound healing. These researchers\(^{18-20}\) have investigated the success of oral wound healing therapy using histology for tissue re-epithelialization and microscopy images for wound closure.

Rinastiti et al\(^{21}\) studied gingival wound healing in rabbits. They examined gingival biopsies in different days after injury and found that epithelial layer, fibroblast number and neo vasculogenesis and collagen formation were decreased, and leukocyte infiltration increases in wound group. Eroglu et al\(^{22}\) studied gingival wound in rats. Their histological findings revealed that collagen fibers, epithelial formation scores decreased, and the inflammation score increased in wound group. In our study, burn injury increased the MDA and MPO levels and also decreased the GSH, epithelization, FGF and EGF expression. Gallic acid treatment improved these scores (Table I, Figure 1). Our histopathological results showed that degeneration in the gingival epithelium, loss of integrity in epithelial and connective tissue fibers, edema and inflammatory cells were observed in the burn group.

Table I. Biochemical and histological parameters of burn and burn+gallic acid groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>n</th>
<th>Median (Min-Max)</th>
<th>Mean Rank</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>Burn</td>
<td>10</td>
<td>49.98 (35.39-65.45)</td>
<td>14.30</td>
<td>p=0.004</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>37.0 (23.78-45.89)</td>
<td>6.70</td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>Burn</td>
<td>10</td>
<td>1.53 (0.98-1.69)</td>
<td>14.75</td>
<td>p=0.001</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>0.69 (0.32-1.54)</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>Burn</td>
<td>10</td>
<td>4.29 (3.27-6.57)</td>
<td>6.60</td>
<td>p=0.003</td>
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<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>7.26 (4.19-9.85)</td>
<td>14.40</td>
<td></td>
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<tr>
<td>Epithelization</td>
<td>Burn</td>
<td>10</td>
<td>2.00 (1.00-4.00)</td>
<td>7.30</td>
<td>p=0.011</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>4.00 (2.00-4.00)</td>
<td>13.70</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>Burn</td>
<td>10</td>
<td>4.00 (0.00-4.00)</td>
<td>12.10</td>
<td>p=0.194</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>3.00 (1.00-4.00)</td>
<td>8.90</td>
<td></td>
</tr>
<tr>
<td>Leukocyte infiltration</td>
<td>Burn</td>
<td>10</td>
<td>3.00 (2.00-4.00)</td>
<td>12.00</td>
<td>p=0.231</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>2.50 (1.00-4.00)</td>
<td>9.00</td>
<td></td>
</tr>
<tr>
<td>FGF expression</td>
<td>Burn</td>
<td>10</td>
<td>2.00 (1.00-4.00)</td>
<td>7.75</td>
<td>p=0.030</td>
</tr>
<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>3.50 (2.00-4.00)</td>
<td>13.25</td>
<td></td>
</tr>
<tr>
<td>EGF expression</td>
<td>Burn</td>
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<td>p=0.018</td>
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<tr>
<td></td>
<td>Burn+gallic acid</td>
<td>10</td>
<td>4.00 (2.00-4.00)</td>
<td>13.45</td>
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</tbody>
</table>

Malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH), fibroblast growth factor (FGF), epidermal growth factor (EGF).
In burn + gallic acid group, epithelium regenerated, collagen bands settled in a certain order, and the inflammation decreased (Figure 1). Our results were consistent with literature.

Growth factors are responsible for stimulating cell proliferation, wound healing, and cellular differentiation. Epidermal growth factors (EGF) and fibroblast growth factors (FGF) are growth factors involved in wound healing and tissue regeneration. Kim et al. studied EGF role in gingival wound healing and found that EGF strongly promoted epithelial cell repopulation and mildly promoted fibroblast repopulation. Papamanoli et al. studied EGF mRNA level in oral epithelium after surgical periodontal therapy. The authors showed that EGF mRNA level increased in two weeks after surgery during wound healing period. Fujihara et al. stated that FGF-2 treatment accelerated the healing of skin wounds and the function as a negative regulator of inflammation during periodontal regeneration and healing. EGF expression was observed in fibroblast cells

Figure 1. a, MDA value of burn and burn+gallic acid group. (b) Graphical illustration of biochemical and histological parameters. Hematoxylin Eosin staining of the skin sections. c, Burn group: Degeneration (arrow) in the gingival epithelium, loss of integrity (star) in epithelial and connective tissue fibers, and areas of edema and inflammatory cells (arrowhead) were seen in abundance. d, Burn + gallic acid group: microscopic papillae areas of the gingiva were invaginated towards the epithelium (arrow) and secondary papillae (arrowhead) were formed. Collagen bands settled in a certain order and the inflammation (star) decreased significantly. FGF and EGF immunostaining of the skin sections. e, Burn Group: FGF expression was positive in fibroblasts (arrow) and connective tissue cells (arrowhead). f, Burn+gallic acid Group: FGF expression was positive in blood vessels (arrowhead) and fibroblasts (arrow). g, Burn Group: EGF was positive in fibroblast cells (arrow) and vascular endothelial cells (arrowhead). h, Burn+gallic acid Group: Intense EGF expression was observed in the connective tissue (star) and basement membrane (arrow).
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and vascular endothelial cells. In burn+gallic acid group, EGF expression was increased in the connective tissue and basement membrane. FGF activity in burn group was observed in fibroblasts and connective tissue cells. In burn+gallic acid group, FGF expression was increased in blood vessels and fibroblasts.

Conclusions

In conclusion, we can suggest that gallic acid has the potential for better healing outcomes in oral wounds. Gallic acid seems to have promising therapeutic efficacy to enhance oral wound healing.

Conflict of interest

All authors have nothing to disclose. There is no conflict of interest.

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Ethics Approval

Ethical approval of the study was obtained from Dicle University Animal Experimental Local Ethical Committee, Turkey.

Informed Consent

Not applicable.

Availability of Data and Materials

All data in this study was included in the manuscript.

Authors’ Contributions

All authors contributed equally to manuscript drafting, writing, data collection, conceptualization and observation.

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