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ORIGINAL ARTICLE

The effect of Salvadora persica ethanolic extract on oral tissue healing in rats: An in vivo study

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KEYWORDS

Salvadora persica;
Miswak;
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Abstract *Introduction:* Invasive surgical procedures in the oral cavity inevitably cause trauma to the soft and hard tissues. The healing process in the oral cavity tissue occurs in a complex manner involving different types of cells, maturation process, and the time of healing. *Salvadora persica* (miswak) has been found to exert various positive effects on the oral cavity, including antimicrobial, anti-gingivitis, anti-cariogenic, gingival healing, and teeth whitening properties. This study aims to investigate the potential of miswak as an adjunctive therapy in promoting wound healing.

Materials and methods: 30 live Sprague-Dawley rats were used in this study. The rats' mandibular first molar tooth was extracted, and an incision wound was made on the tongue. The extraction socket and incision wound were irrigated using normal saline and different concentrations of locally processed miswak plant extracts (0.05%, 10%, and 20%) for 7 days. The rats were sacrificed for gross examination of the tooth socket and tongue healing. Both soft tissue and alveolar bone were examined microscopically.

Results: Complete closure of the incision wound was observed on all rats' tongues; miswak groups showed better wound healing than control and placebo groups in the oral mucosa overlying the alveolar bones. 0.05% and 20% miswak extracts showed prominent wound healing effects in the

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sagittal sections of the tongue, with moderate formation of connective tissue under the wound site and notable wound contraction. The 20% miswak extract group showed the highest percentage of healed oral mucosa on the alveolar bone and higher bone deposition at the alveolar base.

Conclusion: A concentration of 20% miswak extract enhances the initial phase of wound healing both in oral soft and hard tissues. Miswak extract at this concentration was not toxic to the tissues and had potential therapeutic effects in oral tissue healing.

1. Introduction

Invasive surgical procedures in the oral cavity inevitably cause trauma to the soft and hard tissues. These procedures may range from simple tooth extraction to surgeries that create defects such as the excision of oral cancer tissues. Healing of the soft and hard oral tissue occurs in a complex manner involving different types of cells, maturation process, and timing of healing (Politis et al., 2016). Delayed wound healing indirectly reduces the patient's oral health-related quality of life (Ruvo et al., 2005). The extra pain, cost, and time consumed to treat post-operative complications associated with delayed healing processes such as alveolar osteitis and localized osteomyelitis could be spared if the biological healing process can be improved clinically. This is beneficial, especially in patients with compromised wound healing (Guo and DiPietro, 2010). Therefore, optimization of the postoperative healing process is an integral aim in surgery.

Salvadora persica also known locally as miswak has been widely studied and reported to have various positive mechanical and chemical effects on the oral cavity. Among the studied effects of miswak are its anti-microbial, anti-gingivitis, anti-cariogenic, gingival healing, and teeth whitening properties (Nordin et al., 2020).

Extracts from different parts of miswak have been reported to contain a wide variety of organic and inorganic compounds. Among the phytochemical compounds reported in miswak are phenol, carbohydrates, terpenes, sterols, glycosides, saponins, flavonoids, alkaloids, tannins, and benzyl derivatives. Other compounds identified include fluoride, chloride calcium, phosphorus, silica, sulfate, ascorbic acid, thiocyanate, and nitrate (Aumeeruddy et al., 2018). Miswak is a well-known anti-microbial agent of the oral cavity that may aid in the healing of gingival wounds. As a result, its effect on the gingival wound healing process has been studied in literature (Dorri et al., 2012; Nordin et al., 2020).

Considering the widely reported positive effects of miswak on oral health, this pilot study aims to investigate the potential of miswak as an adjunctive therapy in promoting wound healing following hard and soft tissue injuries in the oral cavity after intraoral surgical procedure based on clinical appearance and qualitative histological assessment in vivo using Sprague-Dawley rats.

2. Materials and methods

This research was approved by the Institute of Animal Care and Use Committee (IACUC)(ID: IIUM/IACUC-2018 (27)).

2.1. Sample size calculation

Using PS-Power and Sample Size Calculation software, 7 rats were used in each group. Previous data indicate that the response within each subject group was normally distributed with a standard deviation of 9.1 (Dorri et al., 2012). 30 live Sprague-Dawley rats weighing around 250–300 g were used for this study. However, throughout the course of the experiment, 10 rats died due to aspiration during animal surgery. The remaining rats were redistributed to 6 per group before commencing the irrigation procedure.

2.2. Preparation of miswak stem

One kilogram of miswak stems (Karachi, Pakistan, Al khair CQW, Pakistan) was crushed using a mortar and pestle before cut into smaller pieces and crushed into a powder using a grinder. The powdered stems were weighed then soaked in absolute ethanol (Merck, Germany) to extract the phytochemical components. The macerate was filtered thrice using Whatman filter paper No. 1 and evaporated using rotary evaporator (Buchi Rotavapor R-215, Flawil, Switzerland) at 60 °C (Buchi Vacuum Controller V-855, Flawil, Switzerland). The crude extract was pooled and weighed and final yield of the extract was calculated. 75.93 g (7.593%) of ethanolic extract was yielded. The extract was left to dry in the drying cabinet at 37 °C. 0.05%, 10%, and 20% concentrations of miswak extract were prepared using 10% dimethyl sulfoxide (DMSO).

2.3. Surgical procedure

The rats were divided into five groups. Rats were administered with an intraperitoneal anesthesia injection (ketamine/xylazine) at 0.1 ml/100 g body weight. Forceps were used to extract the left mandibular first molar, followed by an incision of a 2 mm length wound using a no. 15c surgical blade on the left side of the anterior third of the tongue.

In Group 1 (control), tooth extraction was done without any wound irrigation. In Groups 2, 3, 4, and 5, the wounds were irrigated with 2 ml of normal saline (placebo), 0.05%, 10%, and 20% of miswak extract respectively, twice daily (9 am and 4 pm) for 7 days.

On the 8th day, the rats were sacrificed in an ethyl jar filled with chloroform. The mandible and the tongue were dissected, and a gross examination of the wound healing was done. Clinical parameters observed during the gross examination were soft tissue growth, inflammation, abscess formation, and complete closure of the extraction socket. After examination, the specimens were fixed in 10% formalin.

2.4. Histological slides preparation

The mandibles were immersed in a 10% hydrochloric acid solution for 6 h for decalcification. The specimens were processed in a tissue processing machine (Thermo Scientific Excelsior™ AS Tissue Processor, Waltham, Massachusetts, U.S.) and embedded in paraffin wax the next day (Thermo Scientific HistoStar™ Embedding Workstation, Waltham, Massachusetts, USA. The sections were cut using a microtome (Leica RM2255, Germany) with thickness set at 3 μm. The sections were stained with hematoxylin and eosin using an automated staining machine (Leica Autostainer XL ST5010, Germany). The prepared slides were assessed under a light microscope (Olympus BX51TRF Transmitted and Reflected Light Research Microscope, Japan). The criteria for histological examination were inflammation, fibrosis, epithelial regeneration, bone deposition in the tooth extraction socket and necrosis.

3. Results

3.1. Gross examination of soft tissue

All 5 groups showed complete closure of the wounds on the mucosa of the tongue. Meanwhile, for the gingival mucosa, all miswak groups showed better wound healing than the control and placebo groups. Group 5 presented with the highest incomplete soft tissue formation on the socket and complete closure of the extraction socket. Groups 3 and 4 showed no difference in healing pattern when compared to the control. Group 2 showed the least healing effect with most of the sockets presenting with granulation tissue at day 8 (Fig. 1).

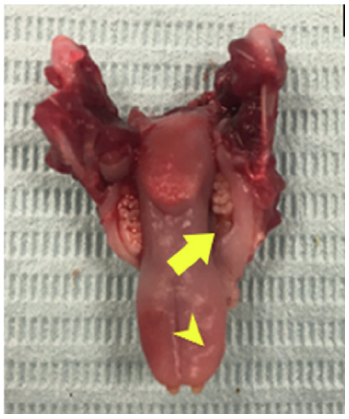

Fig. 1 Gross examination of the specimen. Specimen of mandible and tongue dissected from rat in Group 5 (20% miswak extract). Complete soft tissue coverage is seen at the socket (arrow) with complete healing of the laceration wound of the tongue (arrowhead).

3.2. Histological examination of soft and hard tissue

The sagittal sections of the tongue from rats in the control, 0.05%, and 20% miswak groups showed signs of wound healing with connective tissue formation under the lamina propria, formation of thick epithelium, well-defined papillae, and notable wound contraction. Group 3 presented with the most wound contraction and epithelium formation. Groups 2 and 4 demonstrated scarce connective tissue formation at the wound site and thin epithelium and poorly defined filiform papillae formation (Fig. 2).

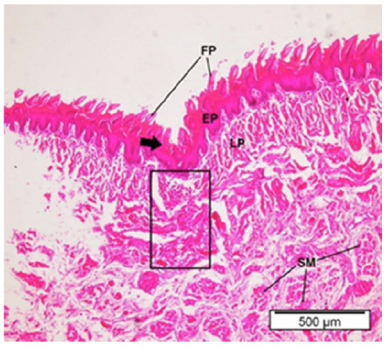

Fig. 2 Sagittal section of a tongue. Sagittal section of rat tongue treated with 20% miswak extract. A notable area of connective tissue is seen (box), along with new epithelium and shortening of the wound edges at the wound site (arrow). (**EP:** epithelium; **LP:** lamina propria; **FP:** filiform papillae; **SM:** skeletal muscle). (Hematoxylin and eosin staining, magnification 40X. Bar = 500 μm).

Histopathological analysis of the sagittal sections of alveolar bones showed notable gingival tissue formation closing the extraction sockets, with all sample groups showing better gingival wound healing compared to control and placebo. A marked formation of granulation tissue was seen filling the

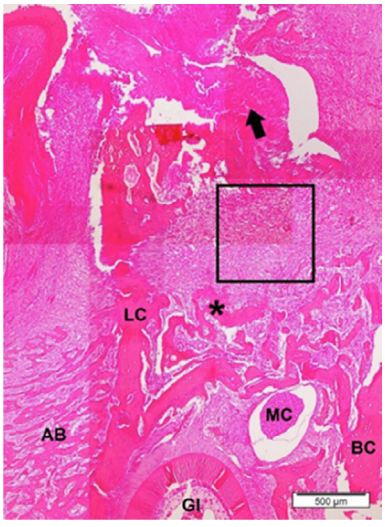

Fig. 3 Sagittal section of extraction socket. Sagittal section of rat mandible treated with 20% miswak extract. Newly formed epithelium (arrow) and bone islands (asterisk) can be seen. The section shows prominent formation of new bone islands. Granulation tissue was minimal (square box). (**AB:** alveolar bone; **LC:** lingual cortex; **MC:** mandibular canal; **GI:** growing incisor; **BC:** buccal cortex). (Hematoxylin and eosin staining, magnification 40X with superimposition of multiple images. Bar = 500 μm).

sockets in all miswak groups. New bone formation was more prominent in the miswak groups compared to the controls, with Group 5 having the highest new bone formation at the base of the extraction socket. Group 1 showed scarce new bone formation and a lack of granulation tissue filling the extraction socket. Overall, Group 5 showed prominent gingival soft tissue growth, granulation tissue formation, and most recent bone development in the alveolar region (Fig. 3).

4. Discussion

Based on gross examination, no difference in tongue mucosa wound healing rate was observed between all groups. No evidence of inflammation, abscess formation, or wound dehiscence was observed on the tongue, implying that miswak at prepared concentrations posed no adverse or harmful effects on the oral mucosa healing process. Gingival mucosa overlying the extraction sockets in the three miswak groups also showed relatively better wound healing compared to the control and placebo groups. This finding is supported by Balto et al. (2015), who reported that application of 5% miswak ethanolic extract on human gingival fibroblasts (HGF) showed 100% cell viability with no cytotoxic effects when assessed using three different cytotoxic assays. However, studies using 0.1% miswak in mouthwash formulation showed cytotoxic effects on macrophage, epithelial, fibroblast, and osteoblast cells. This can be attributed to other ingredients present in the mouthwash formulation other than miswak extract, which may have contributed to cytotoxicity (Mohammadi et al., 2009).

The miswak extract contains active compounds such as alkaloids, sulfur compounds, benzyl nitrite, and thymol that possess bactericidal effects against most periodontal pathogens (Sukkarwalla, A. et al., 2013). The absence of any observed post-operative infection and inflammation in this study may be attributed to the antibacterial and anti-inflammatory nature of the miswak extract. Flavonoids are associated with anti-inflammatory activity, which may reduce chronic inflammation at the wound site, resulting in improved healing time. According to Ibrahim et al. in 2011, three major flavonoids in the miswak extract i.e. apigenin rhamnoglucoside, luteolin glucoside, and rutin significantly reduced serum inflammatory mediators interleukin-1ß, interleukin-6, tumor necrosis factor-α and transforming growth factor- ß1 in rats with carrageenan-induced paw edema.

The wound healing re-epithelialization process was emphasized during histological examination because it is a major sign of tissue regeneration events. In normal wound healing, re-epithelialization is initiated to restore the wounded tissue to its original epidermal structure by means of migration and proliferation of epidermal cells and fibroblasts to the wound site and the formation of a new basement membrane (Cohen et al., 1992).

Distance between wound edges in Group 3 and Group 5 was minimal, indicating higher wound contraction with application of miswak extract compared to the control group. Wound contraction occurs due to the movement and pulling of collagen fibroblasts and myofibroblasts on the wound edge granulation tissue (Swaim et al., 2001). Wound contractions mark the completion of the tissue healing process before the remodeling phase.

Many factors have been shown to influence the healing rate of oral mucosa such as the environment and exposure to saliva bath, re-epithelization process, low inflammation, quicker resolution rate, decreased angiogenic response, and the involvement of different fibroblasts lineage (desJardins-Park et al., 2019). These factors not only contribute to scarless wound healing of the oral mucosa but also result in healed tissue that closely resembles the original and unwounded mucosa. In the current study, besides the lack of scar formation, no tissue necrosis or inflammation was observed in the sections. Hence, the oral tissues were not adversely affected when bathed post-operatively in miswak extract for 7 days.

At the end of the healing process of an extraction socket, there should be a good amount of bone deposition replacing the empty socket and a good barrier of gingival mucosa overlying it. The three phases involved in alveolar socket healing are the inflammatory, proliferation, and remodeling phase. In the current study, the miswak groups showed high epithelium formation. The formation of granulation tissue filling the socket of the extraction site reflects the positive wound-healing process of the socket on day 8, as the replacement of blood clots by the granulation tissue in the socket is expected to happen a week after tooth extraction (Politis et al., 2016).

In the landmark study by Guglielmotti et al. (1985) on histological features in rat alveolar bone healing, the maximum alveolar bone formation in rats takes place at 14 days and the most resorption was seen on day 7 post-extraction (Guglielmotti et al 1985). In the current study, histological assessment done on the 8th day showed highest bone formation at the base of the socket in the group irrigated with 20% miswak extract concentration.

Our study protocol was adopted from Dorri et al. (2012), comparing the effects of pre- and post-operative 0.2% chlorhexidine and 10% miswak irrigation on alveolar bone healing. The authors report that healing of the rat extraction socket was enhanced by 10% with miswak irrigation when compared to chlorhexidine. In the present study, the highest bone deposition at the alveolar base was observed with 20% miswak extract, followed by 0.05% and 10% miswak extract. Hence, higher concentrations of miswak extract may enhance alveolar bone healing.

The accelerated healing effect in the miswak treated groups compared to the control and placebo may be attributed to the presence of vitamin C, flavonoids, saponins, and tannins in the extract, as suggested in a study by Halawany (2012). Vitamin C in miswak aids the healing process through the hydroxylation of proline and lysine, both of which are the by-products that are essential in the formation of collagen in the proliferative phase (Collins, 2009). Additionally, there are no signs of abscess formation and prolonged inflammation in both clinical and histological examinations of the miswak groups. This is in accordance with studies reporting that miswak contains bactericidal chemicals such as alkaloids, benzyl nitrite, and thymol, and anti-inflammatory components such as flavonoids, to combat periodontal pathogens and prevent severe inflammation, respectively (Ibrahim et al., 2011; Sukkarwalla et al., 2013).

5. Conclusion

In conclusion, 20% *Salvadora persica* (miswak) extract enhances wound healing in oral soft and hard tissue. At 20% concentration, miswak extract did not pose any harmful effect

on the oral tissue and has potential therapeutic effects in wound healing. Further clinical investigation can be done with miswak as an adjunct therapy to optimize wound healing in medically compromised patients.

Authors contribution statement

All authors have contributed to the research work: Nor Adilah Harun – contributed to the oral surgery procedures and article preparation and writing. Norazsida Ramli and Abdul Rahman Kazim Azli contributed to the laboratory work and histological slide preparations, prepared results, and article review. Haszelini Hassan contributed to the clinical work and article preparation and review of the paper. Khairul Bariah Chi Adam and Mohd Haikal Muhamad Halil contributed to the article review and editing.

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CRediT authorship contribution statement

Nor Adilah Harun: Conceptualization, Project administration, Investigation, Writing – original draft, Writing – review & editing. **Norazsida Ramli:** Supervision, Investigation, Formal analysis. **Abdul Rahman Kazim Azli:** Investigation, Writing – original draft. **Haszelini Hassan:** Investigation. **Khairul Bariah Chi Adam:** Writing – review & editing. **Mohd Haikal Muhamad Halil:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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