

Effect of *Salvadora Persica* (Miswak) on Alveolar Bone Healing after Tooth Extraction in Rat

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Abstract

Miswak is a teeth cleaning twig made from a twig of the *Salvadora Persica* tree in Islamic hygienically jurisprudence. The beneficial effects of Miswak in respect of oral hygiene and dental health are partly due to its mechanical action and partly due to pharmacological actions. This study aimed to evaluate the effects of *Salvadora Persica* (Miswak) extracts on alveolar bone healing after tooth extraction in rats.

Miswak were grinded into powder. Then two extracts were performed, 96% ethanol extract and aqueous extract. 2ml of the extract from each group were mixed with 4ml of gel as vehicle delivery (2ml pectin 4% & 2ml KI5M) separately. Eighteen rats were used, divided into three groups six rats for each, two experimental and one control groups. All animals were anesthetized, and dental anesthesia was injected locally. The upper right central incisor was extracted, and then sockets were filled with gels containing the Miswak extracts while the control groups filled with the gel alone.

The findings indicate that local application of *Salvadora Persica* would effectively preserve the residual alveolar bone by promoting bone formation in the extraction socket through acceleration of the healing process in rat's teeth socket.

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Introduction

Salvadora Persica (Miswak) tree is a medical plant whose roots have been used by many people in Africa, South America Middle East and Asia.¹ The beneficial effects of Miswak in respect of oral hygiene and dental health are partly due to its mechanical action and partly due to pharmacological actions.² WHO has recommended and encouraged the use of these sticks MISWAK- Siwak as an effective tool for oral hygiene.³ *Salvadora Persica* (Miswak) functions have been evaluated by many researchers. It was been proven as anti-bacterial, anti-mycotic, analgesic and used in endodontic irrigation, mouthwashes, toothpaste and dental plaque inhibiting agent.⁴⁻⁸ Studies have indicated that *Salvadora persica* contain substances that

possess plaque inhibiting and antibacterial properties against several types of cariogenic bacteria which are frequently found in the oral cavity.^{9,10} Farooqi et al. isolated benzyl-isothiocyanate from *Salvadora Persica* root; they claimed to have found saponins along with tannins, silica, a small amount of resin, trimethylamine and a fairly large amount of alkaloidal constituents.¹¹ Ray et al. found B-sitosterol, m-anisic acid, and salvadouria [1,3-Bis-(3- methoxy-benzyl)-urea].¹² Lewis and Elvin-lewis report a high content of minerals in the root: 27.06%.¹³ Miswak shows effective antibacterial activity against wide range of bacteria in -vitro and in vivo.^{4,14} Almas and Zeid examined the immediate antimicrobial effect of a toothbrush and Miswak on cariogenic bacteria (especially on streptococcal mutans and lactobacilli). They evaluate effect of Miswak (chewing stick), Miswak extract, toothbrush, and normal saline on mutans and lactobacilli. The results showed there was a marked reduction of streptococcus mutans among all groups.⁸ Almas et al. assess the antimicrobial activity of eight commercially available mouth rinses and 50% Miswak extract

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against seven microorganisms Mouth rinses containing Chlorhexidine was with maximum antibacterial activity, while Miswak extract was with low antibacterial activity.¹⁰ Fouad et al. studied the effect of mouthwash extracted from *Salvadora Persica* (Miswak) on dental plaque formation. Traces of *S. Persica* 10 mg/ml solution were detected up to 6 h after expectoration; this substantivity is approximate but it gives a comparable gross picture. They demonstrated that *S. Persica* alcoholic extract produced remarkable antibacterial activity in vitro at 10 mg/ml concentration, is well tolerated and safe. As a mouth rinse is less effective than Chlorhexidine in preventing plaque accumulation and more effective than placebo on dental plaque accumulation.⁴

The acute toxicity test (single dose toxicity) is still of considerable importance for the assessment of risk posed by new chemical substances, and for better control of natural and synthetic agents in the human environment.¹⁵ Evaluation of toxicological profile of the Miswak extract by intra peritoneal injection did not cause death among the experimental animals even with a very high dose which was 2.04 g/kg. Therefore; the extract is considered as a well-tolerated substance and the LD50-test is without practical or theoretical relevance.^{15,16} However, the effect of *Salvadora Persica* toward the acceleration of socket healing has not yet been evaluated. Therefore the aim of this study was to assess the acceleration of extraction socket healing following single tooth extraction on rats.

Materials and methods

Plant Material

The *salvadorapersica* (Miswak) sticks were purchased from the market, grounded into a fine powder by using a hammer meal grinder. Concentration of 10 mg/ml of aqueous and ethanol extractions were prepared according to the method described by Fouad et al.⁴

Ethanol extraction

100 grams of dried powder of Miswak added to 2 liters of 95% ethanol then stored at room temperature for 3-7 days. The extracts were filtered and concentrated by using EYELA rotary evaporator. Finally, the concentrated extracts stored in the oven at 50°C until used.^{4,17}

Aqueous extraction

100 grams of dried powder of Miswak was added to 2 liter of sterile distilled water then heated on hot plate with magnetic stir bar for 3 hours. After that, it filtered. The filtered-extract centrifuged at 3500 rpm for 5 minutes and then filtered back. The ice cube prepared from the supernatant stored in the fridge at -20 °C. The resultant was transferred to -70 °C and dry freezing for 2 days to form powdered extract. Lastly the extract was stored at -20 °C until experiment.

Gel formulated and invented by the Faculty of Dentistry and Pharmacy which contain 2ml pectin 4% & 2ml hydroxypropylmethyl cellulose (HPMC), was used as carrier for the Miswak extract. Two ml of each Miswak extracts were mixed with two ml of gel to prepare the Miswak gel.^{4,17}

In-vitro study of miswak release

A 'vial' method was employed for the in vitro release study. 1 ml of Miswak gel were added to 5 ml of distilled water and mixed together. Samples (2.0ml) was withdrawn periodically at an interval of 2 up to 6hour and at 1, 2, 3, 5, 7, 9, 11, and 15 days, each time replacing the sample with the equivalent of fresh distilled water to maintain the same volume. The samples were analyzed by using spectrophotometer at 350nm. The concentration of Miswak is to be calculated from the calibration curve prepared in distilled water. An in vitro drug release was constructed from the data obtained.^{18,19}

Experimental animal

Eighteen adult male *Sprague dawley* rats (7-8 weeks old) with an average body weight of 200-250 grams were obtained from Animal House, Faculty of Medicine. The rats were transferred to the Experiment Animal Laboratory to acclimatize by All animals were bred in the Universiti Teknologi MARA and housed under similar conditions (22 °C room temperature, 40% humidity, and 12 hours daylight cycle). The rats were fed with *ad libitum*, a standard laboratory diet and tap water. Throughout the experiments, all of the animals received human care, according to the criteria outlined in the "Guide for

the Care and Use of Laboratory Animals,” prepared by the National Academy of Sciences and published by the National Institute of Health. The study was approved by the Research Committee on the Ethical Use of Animal in Research (UiTM Care) Universiti Teknologi MARA Ethic No. 600-FF (PT,5/2) 20/7/2009. All animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the national Institute of health. Animals were divided randomly into 3 groups where each groups consist of 6 rats were housed separately in plastic cages.²⁰

Tooth extraction protocol

Eighteen rats were used in this research, right maxillary incisor of the rats was extracted according to process described by Okamoto and de Russo.²¹⁻²³ Prior to the extraction process the rats were anesthetised by intraperitoneally injecting sodium pentobarbital (50 mg/kg body weight). The rats were locally infiltrated with a few drops of 2% lidocaine containing 1: 100,000 epinephrine, delivered into the labial fold,²⁴ to produce local anesthesia and haemostasis.²⁵

After the tooth was extracted, rats were randomly divided into three groups six for each. The socket of the rats was filled with ethanol extract gel, aqueous extract gel according to the groups. While the control group received only blank gel. The health status and healing process of the sockets were periodically monitored after the surgical procedure.

Scarifying of the rats

Two rats were sacrificed by decapitation at 1, 2 and 3 weeks postoperatively from the respective groups by general anesthesia, where sodium pentobarbital (50 mg/kg body weight) was intraperitoneally infiltrated in the rats.²³

Histological study

After sacrificing the animals, the right maxilla was separated from the left by a median sagittal incision. Another incision was made tangential to the distal surface of the incisor to obtain a block containing only the right incisor alveolus. The block was post-fixed in the 4%

paraformaldehyde solution (pH 7.4) for 24 hours at 4 °C, followed by decalcification with 10% EDTA solution (pH 7.4) for four weeks. After routine laboratory processing, the tissue blocks were embedded in paraffin. Serial cross sections at 5µm in a longitudinal direction were obtained and stained with hematoxylin and eosin (Sigma, Aldrich) for histopathologic examination with a light microscope. Ultimately, the results of the newly formed bone tissue have been presented based on the qualitative and quantitative analysis of data.

Microscopic images of the alveolus (final magnification 420x) were superimposed on sheets of paper with a grid containing 100 equidistant points. Four thousand points lying on, the following parameters were histometrically evaluated in the incisors socket by a blinded examiner: Neutrophils, Lymphocytes, Blood vessels. Fibroblasts and. Data were analyzed statistically by the Independent-Sample T Test to compare the effect of different treatments with control Group. SPSS 20 was used to perform the statistical analysis. (*) Indicates the significance level $P < 0.05$, when compared to control group.

Results

In vitro study of miswak release

Result, demonstrate that the invitro release of Miswak gel continue up to 7 days, an important level of Miswak release notice in the first 3 days of the experiment.

Grosse observations

1ST Week after extraction

Macroscopically observation revealed that animal's extraction tooth sockets treated with gel containing ethanol and aqueous Miswak extracts almost close (heal) completely. While the extraction sockets of the control group were still open and healing process still poor which uncompleted healing in all rats (Figure 1).

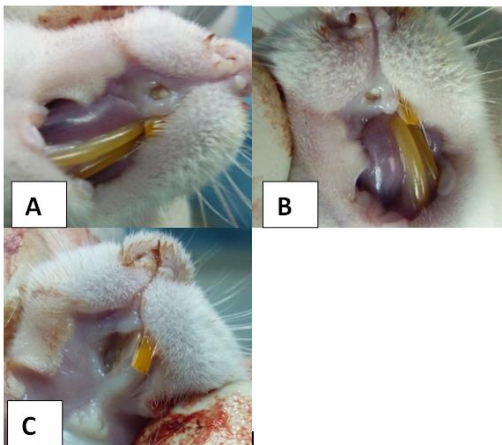


Figure 1. Show the Ethanol Extract socket. A). Aqueous Extract socket; B). Healing almost complete and Control socket. C). Healing incomplete. One week after tooth extraction.

3rd Week after extraction

All animals extraction tooth sockets treated with gel containing ethanol and aqueous Miswak extracts were almost completely closed (healed), Control sockets healing incomplete after three weeks (Figure 2).

Histological Study

1st week

Tooth socket healing in rats 7 days after tooth extraction (Figure 3) demonstrated that sockets treated with gel containing ethanol Miswak extract (A) filled with immature fibrovascular granulation tissue (FVGT) rich in blood vessels with sprinkles infiltration of chronic inflammatory cells, and infilling with woven bone (WB) was observed at the bottom of the socket, note the remnants of residual periodontal ligament (RPL) adherent to the socket margin. Gel containing aqueous Miswak (B), the socket filled with a fibrous granulation tissue (GT), osteoclast-like cells were observed on the surfaces of the bundle bone adjacent to the extraction socket (black arrows), note the acidophilic matrix and osteons formation in mature cellular granulation tissue (blue arrow). While the control group (C), the socket filled with a fibrinous blood clot (BC), immature fibrovascular granulation tissue (FVGT) rich in blood vessels with sprinkles infiltration of chronic inflammatory cells and bundle bone (BB),

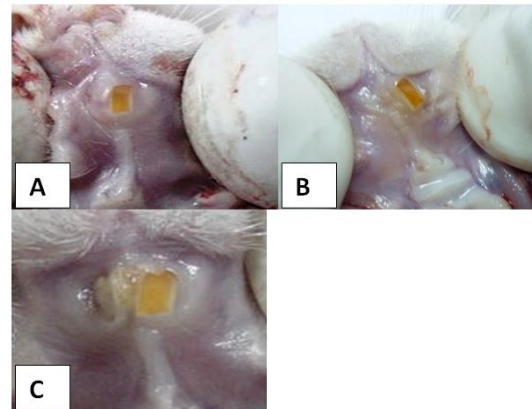


Figure 2. Show the Ethanol A and Aqueous B Extract sockets, healing almost complete and Control socket C, healing incomplete after three weeks.

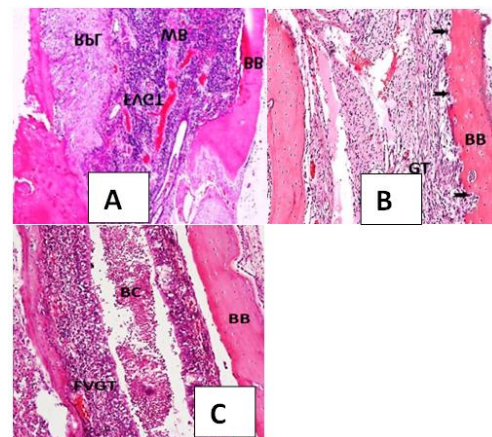


Figure 3. Histological appearances of the extraction sockets during healing. Ethanol A and Aqueous B Extract sockets, Control socket C, 7 days after tooth extraction. H&E staining, original magnification 10X.

3rd weeks

Tooth socket healing in rats 21 days after tooth extraction (Figure 4) demonstrated that sockets treated with gel containing ethanol Miswak extract (A) showing newly formed bone trabeculae (BT) surrounded by osteoblasts (arrows) with blood vessels (BV) and no inflammatory cells observed. Aqueous Miswak (B) showing mature bone trabeculae (BT) surrounded by osteoblasts (arrows), with residual blood vessels (BV) and no inflammatory cells observed. While the alveolus of control animals (C) filled with a fibrous granulation tissue (GT) and no newly formed bone trabeculae observed.

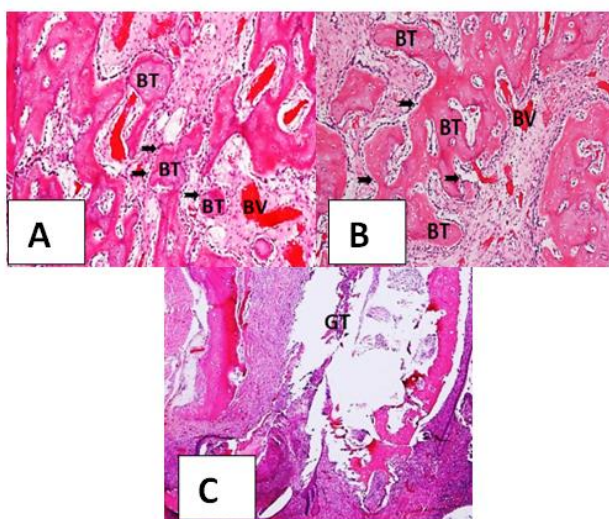


Figure 4. Histological appearances of the extraction sockets during healing. A). Miswak Ethanol and Aqueous B). extracts sockets. Control sockets; C). 21 days after tooth extraction. H&E staining original magnification 10X.

Quantitative results:

Histological study of socket healing after tooth extraction revealed significant differences between experimental Miswak Ethanol extract (MKE), Aqueous (MKAq) and control groups for all the cells. Scores in red were significantly different when the two groups were compared for each variable and experimental time period (Table 1).

Discussion

Alveolar bone defect due to tooth extraction greatly hinders the placement of implant. Therefore, preservation of the alveolar process in areas of tooth loss and adoption of procedures that minimize bone loss or recuperate the desirable alveolar ridge dimensions are important goals in dental practice.²⁶⁻²⁸

Alveolar wound healing provides a suitable model for the study of bone formation in rats and can be considered a sensitive indicator of bone damage under different experimental conditions.^{29,40-43} Healing process of dental alveolus in rats is similar to that in humans in the sequence of events, but occurs more rapidly.^{26,44-46} Rat alveolar healing process takes about one third of the time of human healing process (21 days for rat dental alveolus repair against 64 days in man).²¹ This healing time allows the

realization of a complete study in small time period.

It has been shown that using this herbal medicine MISWAK or its extract would support periodontal health, reduces the accumulation of microbial plaques, bleeding during brushing, controls gingivitis and periodontal diseases.^{7,17,18} However many types of herbal medicine used in wound care treatment and in wounds following tooth extraction like *Andrographis paniculata*, Ellagic acid.⁴⁷⁻⁴⁹ Herbal extract had not been used to enhance healing of extracted teeth. Thus no earlier study found in the literature to compare it with our results. To the best of our knowledge, this is the first study of the effect of MISWAK on bone healing. Thus, this study was performed to determine whether MISWAK extract affects alveolar bone healing in rats. Generally gross observation revealed that socket treated with ethanol and aqueous extracts healed faster than control group. Histologically at day 7, it was possible to observe well organized bone trabeculate in the experimental group, which could not be found in the control group. The quantity of osteoid, immature bone, osteocytes and osteoblasts were significantly higher in the experimental group. Giving support to these data, our histological findings of control group are according to other studies that evaluated the normal healing process of rat alveolus.^{21,22} Taken together; our findings are indicative of acceleration of the healing process due to the implanted Miswak gel in the experimental group. In the 14 days period, the microscopic features of control group are compatible with the normal healing process of dental alveolus in rats at this time period. In the experimental group, more organized bone tissue was observed, and the mature bone quantity was significantly higher in the experimental group showing that the implanted Miswak gel was still promoting acceleration of bone repair. Guglielmotti et al. reported maximum bone formation and maximum alveolar volume at 14 days after extraction in rats.⁵⁰ Histological analyses have suggested that rat alveolar healing is completed by the end of the 3rd week after tooth extraction.⁵¹ At 21 days, clear differences between control and experimental groups were observed in the parameters related to osteoid, immature bone and osteocytes. Presence of mature bone was significantly higher in the experimental group clearly showing that the healing process was in a

more advanced stage. It has been proven that the greatest proportion of bone formation⁴⁹ and the maximum mineral bone density⁵³ take place by the end of the 2nd week. In the present study, the histological analysis of bone healing was carried out at the end of the 2nd and 3th weeks after tooth extraction, thus comprising both the period of maximum new bone formation and the end of the healing process. Quantitative assessment of the cells during healing following incisor extraction in adult rats indicated the healing of the maxillary incisor socket after 21 days post-extraction. Even though the animal used in this study was the rat, the results revealed significant finding associated with local application of Miswak, it could provide new line of therapy to be used in clinical dentistry. Future research may be carried out to improve the uses of this gel in implant and 3rd molars extractions.

The typical decreased vertical facial height of this patient resulted from the congenital.

Conclusions

Histologically socket treated with ethanol and aqueous extracts showed more trabecular bone formations with presence of Osteoblasts cells representing faster healing process than control group. The findings indicate that local application of *Salvadora Persica* would effectively preserve the residual alveolar bone and promoting bone formation in the extraction socket by accelerating healing process in teeth socket of rats.

Declaration of Interest

The authors report no conflict of interest.

Treatment	Week 1	Week 2	Week3
Control (Blank gel)			
1. Neutrophils	351.00 ± 2.39 ^a	125.00 ± 2.06 ^a	18.00 ± 0.41 ^a
2. Lymphocytes	12.83 ± 0.79 ^a	65.33 ± 2.17 ^a	21.33 ± 1.07 ^a
3. Blood vessels	0.83 ± 0.31 ^a	6.00 ± 0.87 ^a	9.00 ± 0.32 ^a
4. Fibroblasts	1.00 ± 0.00 ^a	40.00 ± 2.08 ^a	68.17 ± 2.14 ^a
5. Osteoblast	0.00 ± 0.00 ^a	9.00 ± 1.15 ^a	56.00 ± 1.67 ^a
MKE gel			
1. Neutrophils	212.00 ± 3.73 ^b	45.00 ± 0.33 ^b	8.00 ± 0.32 ^b
2. Lymphocytes	6.17 ± 0.31 ^b	10.00 ± 0.38 ^b	12.00 ± 0.46 ^b
3. Blood vessels	3.00 ± 0.37 ^b	15.33 ± 0.15 ^b	18.00 ± 1.05 ^b
4. Fibroblasts	22.33 ± 4.33 ^b	210.67 ± 4.16 ^b	245.00 ± 4.17 ^b
5. Osteoblast	18.00 ± 0.86 ^b	81.17 ± 2.18 ^b	96.00 ± 2.19 ^b
MKAq gel			
1. Neutrophils	91.00 ± 3.06 ^c	15.00 ± 0.67 ^c	3.00 ± 0.33 ^c
2. Lymphocytes	1.83 ± 0.58 ^c	9.00 ± 0.34 ^b	10.00 ± 0.42 ^b
3. Blood vessels	4.17 ± 0.48 ^b	20.00 ± 0.29 ^c	26.00 ± 1.05 ^c
4. Fibroblasts	44.33 ± 2.26 ^c	270.67 ± 2.46 ^c	312.00 ± 4.28 ^c
5. Osteoblast	33.33 ± 1.20 ^c	115.67 ± 3.14 ^c	141.67 ± 2.17 ^c

Table 1. Histological Study Of Socket Healing After Tooth Extraction In Control And Treated Rats. All Data Expresses As Mean and S.E.M. Mean with Different Superscript are Significantly Difference. $P < 0.05$ are Significant.

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