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ORIGINAL ARTICLE

Comparing the effects of chlorhexidine and persica on alveolar bone healing following tooth extraction in rats, a randomised controlled trial

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Abstract Chlorhexidine is broadly prescribed by clinicians for treating extraction socket wounds; however, studies have reported adverse effects for chlorhexidine. Persica, a herbal antibacterial agent, could be an alternative for chlorhexidine. The aim of this randomised controlled trial was to investigate the effects of persica and chlorhexidine on alveolar bone healing following tooth extraction in rats. Eighteen Wistar rats were randomly allocated to three study groups: 0.2% chlorhexidine, 10% persica and controls (tap water). The rats were mouth-rinsed for 14 days. On day 8, the mandibular right first molars of all the rats were extracted. On day 21, the rats were euthanized and histological slides of their extraction sockets were prepared. The amount of new bone formation and the number of inflammatory cells in the extraction socket for each rat were recorded. Data were analysed using linear regression and Mann–Whitney tests. There was no significant difference between the control group and the intervention groups in terms of new bone formation and inflammatory cell count. The mean new bone formation was significantly higher in the persica group than in the chlorhexidine group. There was a significant association between new bone formation and inflammatory cell count in the entire sample. In conclusion, there were no significant differences between rinsing with tap water and rinsing with 0.2% chlorhexidine and 10% persica in enhancing extraction socket wound healing in rats. Extraction socket wound healing in rats was better enhanced with 10% persica than 0.2% chlorhexidine.

Keywords Chlorhexidine · Persica · Miswak · Bone healing · Extraction

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Introduction

Following tooth extraction, the healing process of the empty socket begins with the formation of woven bone which ultimately remodels and restores the defect [1]. Many factors can influence and enhance or, on the other hand, delay the process of bone healing. These factors include "type of tissue, location and condition of wound, its vascular supply, microbial situation and local and systemic factors" [2–4]. Among these factors, antibiotics and antimicrobial agents, especially when used locally and for prophylaxis, have been shown to enhance the healing process [5–9].

Chlorhexidine is the most common antimicrobial agent used for the prevention of bacterial colonization and, in turn, the enhancement of the socket healing after extraction. Chlorhexidine acts against a broad spectrum of aerobic and anaerobic oral pathogens, is tolerated by the human immune system and does not create resistance [4, 10, 11]. Studies have shown its effectiveness in controlling dental plaque and gingivitis, in enhancing oral wound healing and in the prevention of dental caries and alveolar osteitis [8–10, 12–

17]; however, not all studies support the effectiveness of chlorhexidine in enhancing wound healing in the mouth.

Paunio et al. [17] reported that chlorhexidine can delay the formation of granulation tissue in rats. Similarly, Bassetti and Kallenberger [4] pointed out that post-surgical use of high concentrations of chlorhexidine (higher than 0.5%) delays wound healing in rats. Moreover, the results of an in vitro study indicated that chlorhexidine, at high concentrations, may have cytotoxic effects on human cells [18]. In addition, studies reported adverse effects for chlorhexidine such as an undesirable taste, the discoloration of teeth and restorations, mild stomach complications, mouth dryness and extensive mouth ulceration [10, 12, 14]. These observed adverse effects were directly related to the concentration and duration of taking chlorhexidine.

The adverse effects reported for chlorhexidine and other chemical antimicrobial mouthrinses have attracted attention to herbal mouthrinses which are believed to be safer alternatives. Persica is a herbal mouthrinse. Its main component (90%) is miswak from the *Salvadora persica* tree. Persica also contains mint and yarrow. Miswak is most common in the Middle East and has been used in the form of chewing sticks for tooth cleansing for centuries. Miswak has antiplaque action and many pharmacological properties, including the release of therapeutic chemicals such as fluoride (in large amounts), calcium, vitamin C and tannins [15, 19, 20]. Darout et al. [21] suggested that miswak may have a selective inhibitory effect on the level of certain bacteria in saliva, particularly several oral streptococci species, the main agents in developing dental and periodontal diseases. Moreover, miswak is cheap and its taste is not unpleasant [20]. In contrast, an experimental in vitro study indicated that high concentrations of persica (higher than 0.1%) were toxic to macrophage, epithelial, fibroblast and osteoblast cells [18].

The chemotherapeutic properties of miswak, its low cost and desirable taste would suggest that persica may be an alternative for chlorhexidine in treating post-extraction socket wounds. Although different clinical studies have compared the effectiveness of chlorhexidine and persica (miswak) on periodontal health [22, 23], no known histological study has been carried out to compare the effects of these two mouthrinses on the alveolar bone healing process. The present study aims to investigate the effects of persica and chlorhexidine on alveolar bone healing following tooth extraction in rats.

Materials and methods

The study protocol was approved by the Experimentation Committee of the University of Shahid Sadoughi, Yazd, Iran. Principles of laboratory animal care [24] were followed. Eighteen 3-month-old Wistar rats, nine females weighing 270 g and nine males weighing 350 g, all raised in Shahid Sadoughi Dental School Animal House, Iran, were included in this study. The rats were fed with lab blocks, which mainly contain corn and soy, throughout the study. The sample size was calculated at a 5% level of significance to have a 90% power of demonstrating a significant difference in new bone formation and inflammatory cell count following tooth extraction between the chlorhexidine and persica groups. Using a random number table, rats were allocated to three distinct groups, each consisting of three male and three female rats (Fig. 1). Allocations were concealed until interventions were assigned. Each group was assigned a different mouthrinse; 0.2% chlorhexidine (Shahr Darou Laboratories Co., Tehran, Iran), 10% persica (Pursina Pharmaceutical Co., Tehran, Iran) and tap water (control group). The mouthrinse administrator and outcome assessor were blinded as to the group assignation. For this purpose, mouthrinses were prepared and coded by a dental nurse who was not involved in the implementation of the study. The investigator (MD) administered 5 ccl of the assigned mouthrinse per 10 s using a 5-ccl syringe twice a day, 9 am and 5 pm, for 14 days. Persica was prepared, before each rinse, by mixing with five times the volume of normal saline, as instructed by the manufacturers. The chlorhexidine did not need further preparation. On the eighth day of the experiment, before the administration of the second daily mouthrinse, the rats were placed in a closed chamber containing diethyl ether for

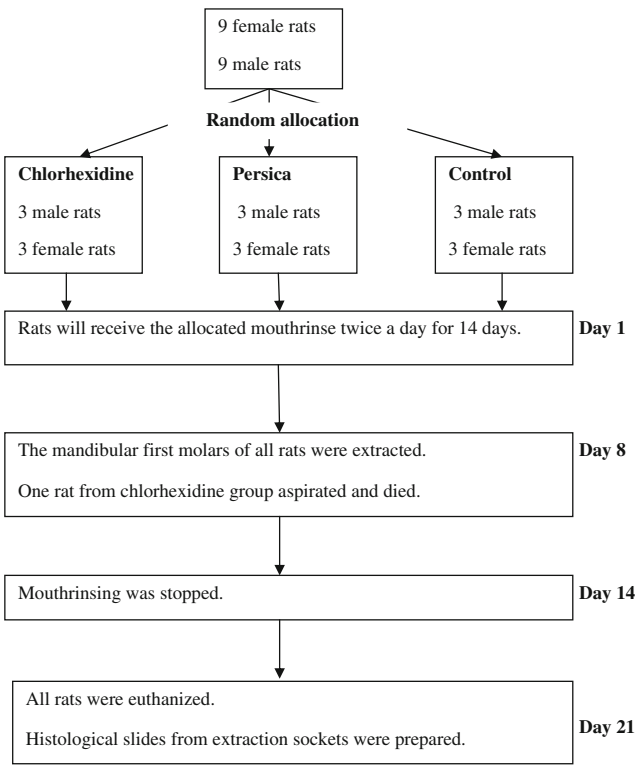

Fig. 1 The study outline

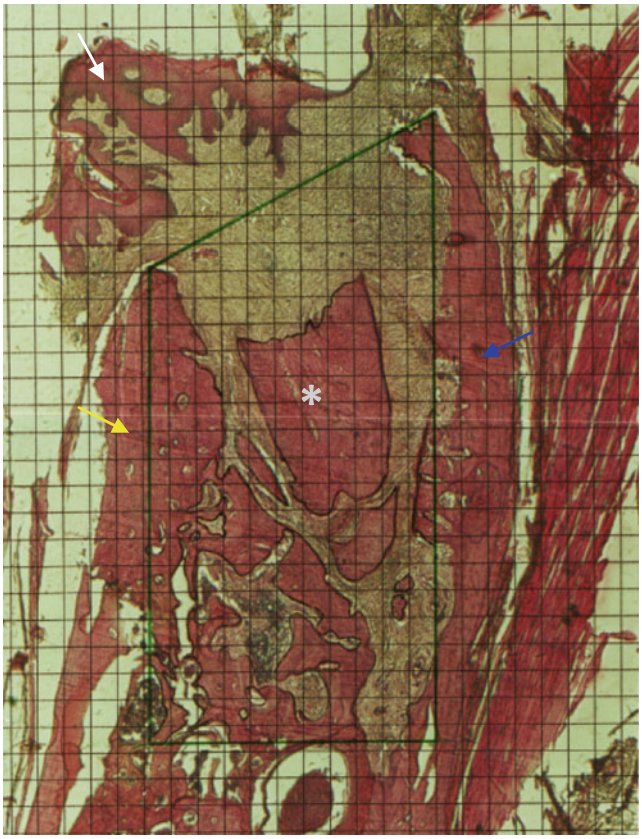

Fig. 2 Microscopic image of the extraction socket in a rat from the control group superimposed with a sheet of transparent paper with a grid containing 100 equidistant points (H&E, ×20). *White arrow*, newly formed epithelium covering the socket wound; *yellow arrow*, lingual cortex; *blue arrow*, buccal cortex; *grey star*, islands of newly formed bone in the extraction socket

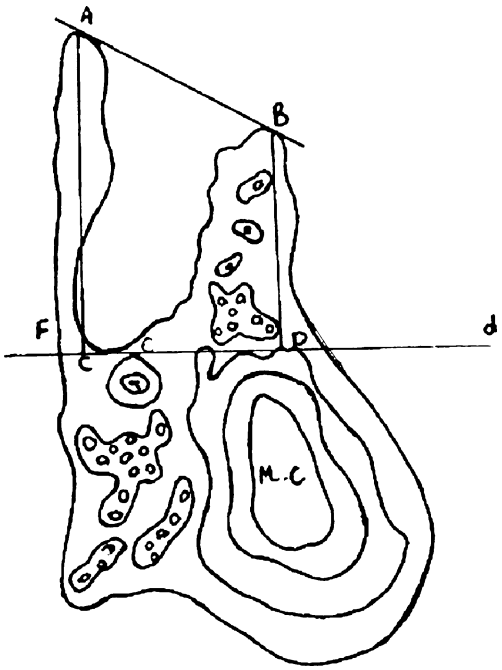

Fig. 3 The outline of extraction sockets in rats [26]. *Point A*, the tip of the buccal cortex; *point B*, the tip of the lingual cortex; *line d*, *horizontal line* drawn from *point E* and *perpendicular* to *line a*; *point C*, the contact point between *line d* and *vertical line* drawn from *point A perpendicular* at *line d*. *Point D*, the contact point between *line d* and *vertical line* drawn from *point B perpendicular* at *line d*. *Point E*, the uppermost point on the mandibular canal. *Point F*, the outermost point on the buccal cortex; *line a*, the *tangent* to the buccal cortex at *point F*

40 s under the supervision of a pharmacologist. Following the sedation, the mandibular right first molars of all the rats were extracted using a pair of child mandibular forceps. One of the rats in the chlorhexidine group aspirated and died. The rats were then observed till fully recovered and the routine daily mouthrinse administration was continued until the 14th day of the trial. Seven days later, during which the rats received no experimental interventions, all the rats were euthanized. For this purpose, the rats were exposed to lethal concentrations of diethyl ether vapour in a closed chamber for 2 min. Following this, the right halves of the rats' mandibles were dissected using blade no. 11. The dissected mandibles were then fixed in a 10% formalin solution. Following fixation, tissues were decalcified in a 2.5% acid nitric solution. Then, the first mandibular molar socket was dissected by applying two buccolingual sections in the mesial and distal of the extraction socket. Later, the bone portion containing the first mandibular molar socket with a thickness of 5–7 mm, was processed and embedded in paraffin. Lastly, using routine histopathologic methods, microscopic slides, each 5 μm thick, were produced and stained with haematoxylin and eosin (H&E). For each sample five H&E sections were prepared. The best H&E section for each sample was selected using the following criteria: the inclusion of intact buccal and lingual cortices, the inclusion of the complete mandibular nerve section, an intact alveolar crest bone and a lack of artefact tissues.

A digital photomicroscope (Nikon) was used to measure the volume fraction of alveolar components by a differential point-counting method [25]. Microscopic images of the alveolus at a final magnification of ×20 were superimposed on sheets of paper with a grid containing 100 equidistant points (Fig. 2). The outline of the alveolar socket was identified as defined by Guglielmotti and Cabrini [26] (Fig. 3). The amount of new bone formation was calculated by the area of new bone formation divided by the area of the whole socket multiplied by 100.

To measure the amount of inflammation, a digital photomicroscope (Nikon) at a final magnification of ×100, was used. In each H&E section, five microscopic fields with the highest number of inflammatory cells (such as lymphocytes, plasma cells and neutrophils) were selected and the number of intact inflammatory cells was counted. Where the surface of the socket was not intact, those inflammatory areas with neutrophils were not selected.

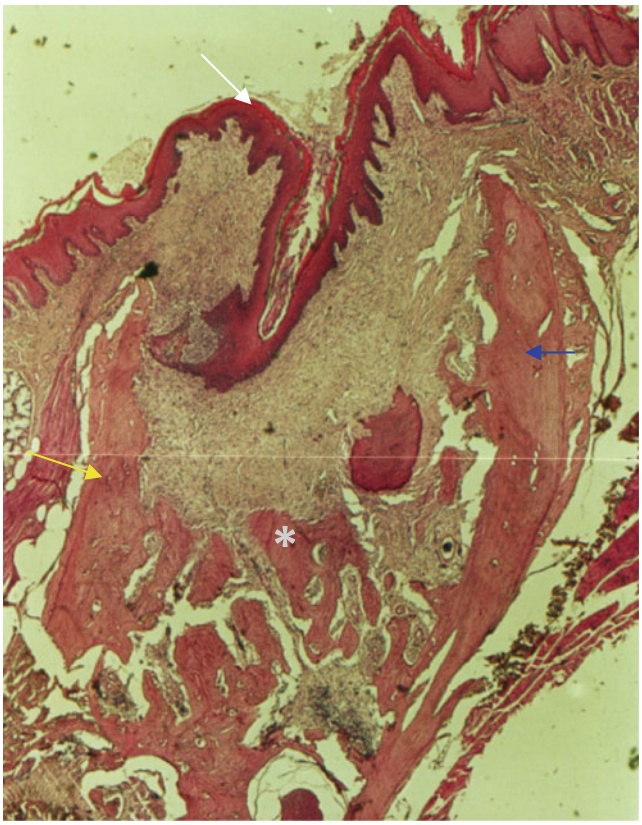

Fig. 4 Microscopic image of an extraction socket in a rat from the chlorhexidine group 14 days after tooth extraction (H&E, ×20). *White arrow*, newly formed epithelium covering the socket wound. *Yellow arrow*, lingual cortex. *Blue arrow*, buccal cortex. *Grey star*, islands of newly formed bone in the extraction socket

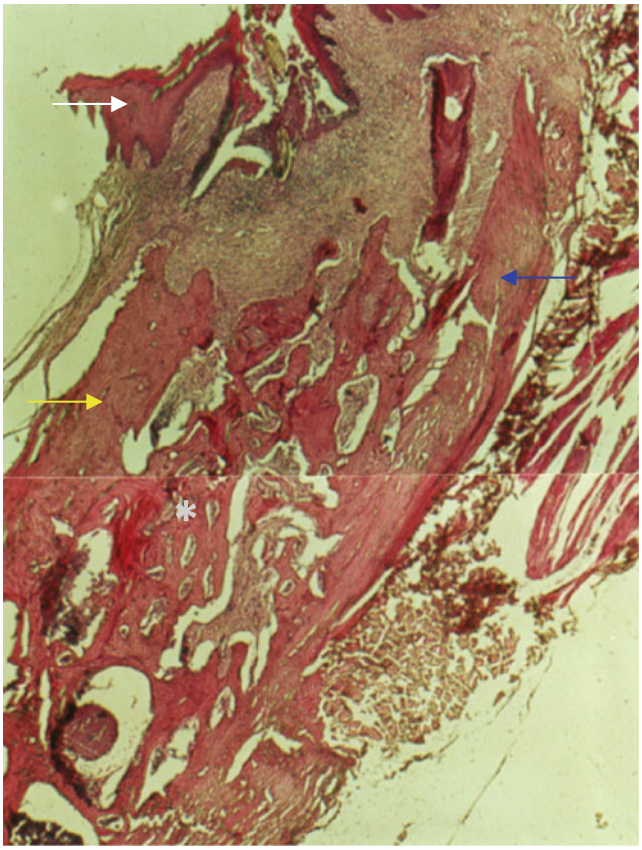

Fig. 5 Microscopic image of an extraction socket in a rat from the persica group 14 days after tooth extraction (H&E, ×20). *White arrow*, newly formed epithelium covering the socket wound. *Yellow arrow*, lingual cortex. *Blue arrow*, buccal cortex. *Grey star*, islands of newly formed bone in the extraction socket

The results from the calculation of the amount of new bone formation and the inflammatory cell count were processed for analysis using the Statistical Package for Social Sciences (SPSS for Windows, version 14.0/PC; SPSS, Chicago, IL, USA). The main outcome variables, new bone formation and inflammatory cell count, were treated as continuous variables. The frequency distributions of the main study outcomes were not normally distributed. The association between the main study outcomes were tested using linear regression analysis. The intergroup differences were investigated using the Mann–Whitney test.

Results

The persica and control groups each included six rats. The chlorhexidine group included five rats.

Histological findings

In the chlorhexidine group, the extraction socket was epithelialised in three cases. The trabeculae of the bone were predominantly evident in the apical third of the socket in all cases. Subperiosteal bone formation was observed in three cases. Intertrabecular connective tissue was present in two cases and it was immature (Fig. 4).

In the persica group, the epithelialisation of the socket wound surface was observed in four cases. Trabecular bone was predominantly evident in the apical third and the internal surface of the buccal and lingual walls. In five cases, the entire socket appeared to be filled with neoformed trabecular bone. Subperiosteal bone formation was present in four cases. In all cases but one the intertrabecular connective tissue was mature (Fig. 5).

In the control group, the socket wound surface was epithelialised in three cases. Neoformed bone was predominantly evident in the apical third and internal surface of the buccal and lingual walls. In one case, the entire socket was filled with trabecular bone. In all cases the intertrabecular connective tissue was mature (Fig. 6).

In all three groups, inflammatory cells were particularly present in the apical third of the socket. In cases in which the socket surface was not intact, inflammatory cells were scattered as focal points.

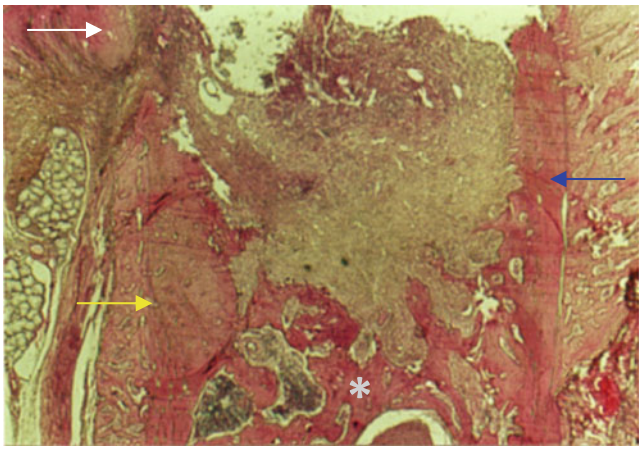

Fig. 6 Microscopic image of an extraction socket in a rat from the control group 14 days after tooth extraction (H&E, ×20). *White arrow*, newly formed epithelium covering the socket wound. *Yellow arrow*, lingual cortex. *Blue arrow*, buccal cortex. *Grey star*, islands of newly formed bone in the extraction socket

Histometric findings

The main study outcomes, new bone formation and inflammatory cell count were not normally distributed. The mean new bone formation was highest in the persica group (51.1, SD=9.1) and lowest in the chlorhexidine group (35.8, SD=9.5). Similarly, the persica group (49.2) and the chlorhexidine group (39.9) had the highest and the lowest median values for new bone formation, respectively (Table 1). The mean inflammatory cell count was highest in the chlorhexidine group (1075.0, SD=569.5) and lowest in the persica group (654.0, SD=502.4). The highest median values for inflammatory bone formation were observed in the chlorhexidine group (982.0) and the lowest median values were observed in the persica group (602.5) (Table 1).

The mean new bone formation values between the chlorhexidine and persica groups were significantly different ($P<0.05$); however, there was no significant difference between the mean new bone formation in the control group and the two intervention groups ($P>0.05$). In relation to the inflammatory cell count, the intergroup differences between the control group and the chlorhexidine ($P=0.20$) and persica ($P=0.63$) groups were not significant. Furthermore, there was no significant difference in inflammatory cell count between the chlorhexidine and persica groups ($P=0.33$). There was a significant association between new bone formation and inflammatory cell count in the entire sample ($p<0.001$). The regression coefficient for this association was 19.3 (95% CI, 4.5, 43.2).

Discussion

There were no significant intergroup differences between tap water (control) and two antiseptic interventions, namely chlorhexidine and persica, in terms of inflammatory cell count and new bone formation in the extraction sockets in rats. This finding is in contrast with findings from other studies which reported a delaying effect for antiseptic mouthrinses, such as chlorhexidine, in healing wounds in animal studies [4, 17]; however, in these studies higher concentrations of chlorhexidine (higher than 0.5%) were used whilst in the present study 0.2% chlorhexidine was administered. Sanchez et al. [27] showed that, despite cytotoxic effects of chlorhexidine on tissue culture fibroblasts in vitro, lower concentrations of antiseptic mouthrinses such as chlorhexidine diacetate 0.005% and 0.05% could benefit wound healing in dogs as compared with normal saline [27]. On this basis, it is speculated that lower concentrations of chlorhexidine and persica could better enhance wound healing. Furthermore, it is shown that the initial delaying effect of high concentrations of chlorhexidine mouthrinse may fade with time. Saatman et al. [28] indicated that although at days 6 and 9, 4% chlorhexidine mouthrinse as compared with normal saline, could result in a slight delay in the healing of incisions and abrasions surgically induced in male guinea pigs; these differences

Table 1 Distribution of new bone formation percentage and inflammatory cell count in the control, chlorhexidine and persica groups

		New bone formation (%)			Inflammatory cells count (*N*)		
		Control	Chlorhexidine	Persica	Control	Chlorhexidine	Persica
Mean		43.8	35.8	51.1	736.0	1075.0	654.0
Median		42.5	39.9	49.2	798.5	982.0	602.5
Standard deviation		13.7	9.5	9.1	667.0	569.5	502.4
Minimum–maximum		28.2–63.8	25.1–46.2	39.8–62.3	99.0–1609.0	639.0–2041.0	155.0–1339.0
Percentiles	25	30.3	25.7	43.6	243.0	654.0	195.5
	50	42.5	40.0	49.2	437.5	982.0	602.5
	75	57.3	43.8	61.2	1558.8	1544.0	1096.8
Number of subjects		6	5	6	6	5	6

tend to disappear by day 21 [28]. This and the fact that adverse effects for antiseptic mouthrinses are related to their concentration may partly explain the variations in the results reported by different animal and human studies on the effects of antiseptic mouthrinses [4, 8–10, 12–18, 27, 28]. Based on this discussion, it is concluded that tap water can be used as an alternative for 0.2% chlorhexidine and 10% persica to enhance alveolar bone healing following tooth extraction in rats; however, it is likely that lower concentrations of chlorhexidine and persica may better enhance wound healing than tap water.

Interestingly, although studies have reported stronger antibacterial activities for chlorhexidine compared to miswak [29], the present study showed that 10% persica was more effective in enhancing bone healing than 0.2% chlorhexidine. New bone formation in the extraction alveolar sockets in rats was significantly better enhanced in the persica group than in the chlorhexidine group. Histological findings were also in favour of 10% persica. Wound healing indicators, including extraction socket surface epithelialisation, intertrabecular connective tissue development and maturation, and subperiosteal bone formation were enhanced in the persica group and were delayed in the chlorhexidine group as compared to the control group. Although the inflammatory cell count was higher in the chlorhexidine group, this difference was not significant. Considering the significant association between inflammatory cell count and new bone formation in the study, it is speculated that this difference would be significant if a bigger sample size was employed. These findings suggest that there is a trend in favour of 10% persica in enhancing extraction socket wound healing in rats as compared to 0.2% chlorhexidine.

Studies indicated adverse effects for chlorhexidine and persica. Mobacken and Wengstrom [30] noted the reduced tensile strength of sutured skin wounds in rats when chlorhexidine disinfection was employed. Moreover, rinsing with chlorhexidine after oral surgical operations, especially surgery in which bone is exposed, may result in the delay and disturbance of wound healing [4]. Few studies have also reported the toxic effects of persica on cells [18]. In the present study, 10% persica was found to be more effective than 0.2% chlorhexidine. Therefore, it can be concluded that the adverse effects of 10% persica on extraction wound healing in rats were less than those of 0.2% chlorhexidine.

In the present study, 0.2% chlorhexidine was used as a comparison group for 10% persica, since it is the gold standard antibacterial agent for oral diseases [31–33]. chlorhexidine has been widely used for years around the world, in some places as an over-the-counter oral product, in many different forms such as mouthrinse, varnish, gel, dentifrice and chewing gum [31–33]. Chlorhexidine mouthrinses are available in two concentrations, 0.12% and 0.2%. Recent studies have indicated that when similar volumes of two concentrations of chlorhexidine were used, 0.2% chlorhexidine was more likely to show better results than 0.12% chlorhexidine mouthrinses [34–36].

One rat from the chlorhexidine group was lost to follow-up due to aspiration. It was not possible to replace the lost rat as the other rats had already been treated with their assigned mouthrinses for 1 week. This may have negatively affected the power of the results. Considering the evident differences between the main study outcomes in the different study experimental groups, it can be speculated that a bigger sample size, which would take dropouts into account, could have led to more significant results in the present study.

In the present study, the time point of 14 days following extraction was considered optimal for the histological and histometric assessment of bone healing. This period of time was chosen as studies have shown that maximal bone formation and maximal alveolar volume occurs on the 14th day after tooth extraction in rats [26].

In conclusion, there were no significant differences between rinsing with tap water and rinsing with 0.2% chlorhexidine and 10% persica in enhancing extraction socket wound healing in rats. Extraction socket wound healing in rats was better enhanced with 10% persica than 0.2% chlorhexidine. Additional clinical trials are required to further investigate the effectiveness of different concentrations of persica and chlorhexidine on the wound healing process in the mouth.

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Conflict of interest The authors declare that they have no conflict of interest.

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