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Original Research Article

Histological evaluation of wound healing by topical phenytoin application on extraction socket: an *in vivo* study in albino Wistar rats

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Abstract – Objective: To investigate the effects of topical phenytoin application on wound healing of extraction socket of albino Wistar rats. **Materials and methods:** Thirty-six albino Wistar rats were randomly divided into Group A (control), B (1% phenytoin mucoadhesive paste), and C (mucoadhesive paste without phenytoin). All animals were anesthetized followed by first molar extraction. Group A did not receive any agent, whereas, Group B and C received daily application of their respective pastes. Four animals from each group were sacrificed on day 7, 14, and 21 and analyzed histologically. **Results:** No significant difference was found in blood vessel count among the groups. Statistical increase in percentage of fibroblast in Group B was found as compared with other groups at day 7, whereas, no significant difference was found among groups at day 14. Day 21 showed significant decrease in percentage of fibroblasts in Group B (p-value < 0.001) as compared with Group A and C. Increase in percentage of bone trabeculae in Group B was observed on day 7, 14, and 21 with a significant difference (p-value < 0.05) as compared to Group A and C. **Conclusion:** Topical application of phenytoin promoted fibroblast proliferation and bone trabeculae formation during healing of extraction socket in rats.

Introduction

The history of tooth extraction dates to 5000 BC by Sumerians. The earliest Egyptian, Hesi-Re was the first dentist to perform tooth extractions around 3000 BC [1]. The healing of the extraction wound follows the basic model of wound healing and involves the overlapping of various events like blood clot establishment, inflammatory responses, regeneration of connective tissue, and remodeling [2].

Wound healing is a prominent health-care problem in today's medical practice [3]. Many agents have been investigated in the past to promote oral wound healing and decrease post-operative complications [4–6] of which one such agent is phenytoin [7,8].

Phenytoin is used as an antiepileptic agent and was introduced by Merritt and Putnam [9]. Its use as a wound healing agent has been investigated for the past 73 years [10] in experimental corneal [11] and chronic wounds, leg ulcers, leprosy wounds, war wounds, skin graft sites [12], various cutaneous, diabetic, decubitus ulcers, burn wounds [13], and fistulas [14]. Studies demonstrated the usefulness of topical phenytoin in a variety of soft [11] and hard tissue wounds [15].

The effect of phenytoin on incisional wounds in the skin [13] and second degree burn wounds of rats showed significant wound healing. Phenytoin overall showed increased vascularization, collagen content, better epithelization with increased levels of VEGF (vascular endothelial growth factor), and TGF-β (transforming growth factor-β) [16].

Effect of phenytoin administration has also been studied on oral wound healing. In 1958, Shapiro reported a study in which oral phenytoin was given prophylactically to patients that were to undergo gingival surgery. Patients treated with phenytoin not only showed enhanced healing, but there was also reduced postoperative pain [8,10]. Furthermore, a recent study showed that topical phenytoin application caused increased palatal wound healing when compared to control [17].

The use of topical application of phenytoin excludes the effects associated with systemic administration [7,8,18].

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Various studies concluded that phenytoin not only decreased the healing time [13] and edema but also showed decrease in bacterial load [16] and inflammation [19] along with possible facilitation of nerve regeneration [18].

Phenytoin acts on fibroblasts and affects their proliferative, synthetic, and migratory activity (chemotaxis and chemokinesis) and cause collagenase inhibition [14]. Phenytoin also increases the number and sensitivity of epidermal growth factor receptors [10] along with keratinocyte motility [18] and proliferation [20].

Angiogenesis occurs along with fibroplasia. The effect of phenytoin on endothelial cells is not well established [8]. The proposed mechanism is that phenytoin has its effect on nerve regeneration by which it causes neovascularization [18].

Histologically, biopsies of phenytoin treated open wounds showed mononuclear cells, eosinophils, increased fibroblasts, collagen deposition, neovascularization, and re-epithelization. It was suggested that phenytoin had a role in chemokinesis. Phenytoin was also suggested to act as a chemoattractant and a potent mitogen for the recruitment of cells, fibroblast proliferation, and angiogenesis [18].

In 1984, a paper was published by The Food and Drug Administration (FDA) office of Orphan Products Development, which listed phenytoin as a potentially useful agent in wound healing [11] and to be judged in a clinical trial [2].

The present study used 1% phenytoin mucoadhesive paste that has not been tested previously for the healing of extraction socket. Moreover, to the author's knowledge, no *in vivo* study on extraction socket has been conducted till day. This study not only analyzed angiogenesis and percentage of fibroblast cells but also the percentage of formation of bone trabeculae caused by topical phenytoin application.

Materials and methods

Formulation of mucoadhesive paste

Oral Mucoadhesive paste was made by the addition of gelatin, pectin, and sodium carboxymethylcellulose to the plastibase. All the ingredients were bought from VWR International Ltd., Singapore and the formulation was carried out at Interdisciplinary Research Center in Biomedical materials (IRCBM), COMSATS University Islamabad, Lahore Campus, Pakistan.

Plastibase was made by gradual addition of pre-heated liquid paraffin (80 °C) to polyethylene. The mixture was continuously stirred (200 rpm) and heated up to 130 °C until a viscous gel was achieved. Liquid paraffin was continued to be added to this mixture until the ratio of 2:1 of liquid paraffin to polyethylene was attained.

This mixture was then poured in a glass jar, already cooled by ice and water to yield plastibase gel.

Weight of sodium carboxymethylcellulose, pectin, and gelatin powders were measured by digital analytical balance (ATX 224 UniBloc, Shimadzu Corporation, Japan). Mucoadhesive paste was made by the gradual addition of sodium carboxymethylcellulose (16.6%) followed by pectin (25%), and lastly gelatin powder (8.3%) to plastibase gel (50%) with continuous stirring (200 rpm) to obtain a homogenous base [21].

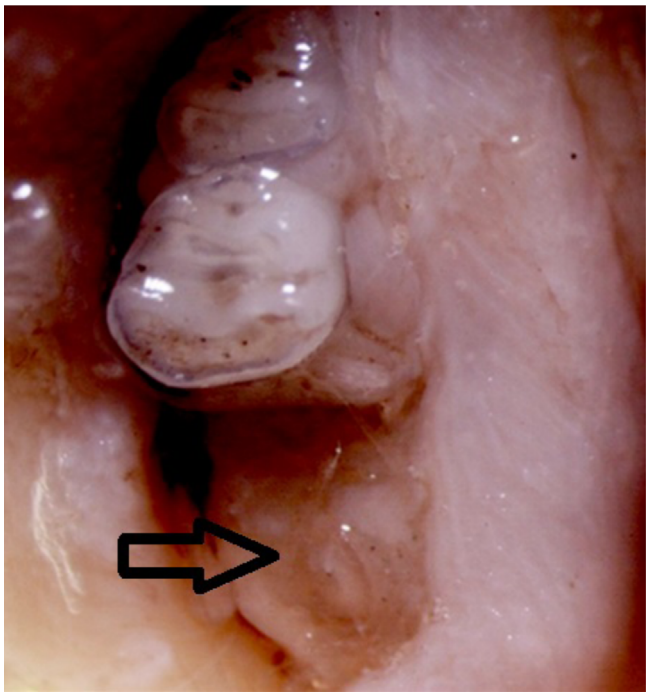

Fig. 1. Extraction socket of left first mandibular molar on day 7. Epithelial coverage over the extraction socket is evident (black arrow).

Pure powder (99%) of phenytoin (Alfa Aesar, USA) was used to make 1% phenytoin mucoadhesive paste for this study [22] and was stored in refrigerator (4 °C) till use.

Procedure

The study protocol was approved by the Ethical Committee of Postgraduate Medical Institute, Lahore, Pakistan (PGMI/AMC/LGH/Research No. 00-41-S-2017/Dated/21-04-2017) and the Advanced Studies and Research Board of the University of Health Sciences, Lahore, Pakistan (No: UHS/Education/126-18/598, dated 06-02-2018). The study followed an experimental design performed *in vivo* in a rat model. The sample size was calculated by the following formula keeping the power of study to 90% and level of significance equal to 5%.

$$n_1 = \frac{(\boldsymbol{Z}_{1-\beta} + \boldsymbol{Z}_{1-\omega 2})^2 (\boldsymbol{\sigma}_1^2 + \boldsymbol{\sigma}_2^2)}{(\boldsymbol{\mu} 1 - \boldsymbol{\mu} 2)^2}.$$

The calculated sample size was 4 [13] in each subgroup with a total of 12 animals in each of the three groups.

Hence, thirty-six albino Wistar rats (35 days old) [23] of either sex, weighing 180–200 g [24], were procured from the University of Veterinary and Animal Sciences, Lahore, Pakistan for this study. Animals that were pregnant or died during the study period were excluded [25]. All were individually housed in a climate-controlled environment and provided with food (pasty chow) and water *ad libitum* at the animal house, Post Graduate Medical Institute, Lahore, Pakistan. All animals used in this study were handled with the international, natural, and institutional

guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care [26]. All animals were kept in a well-ventilated room at an ambient temperature of 28.0 ± 2.0 °C and humidity (60 ± 10%) under 12 h light/dark cycles.

Following acclimatization for one week, the experiment was commenced. Rats were randomly divided into Group A, B, and C with 12 animals in each group ($n = 12$) with the help of an online research randomizer application [27]. Group A included the animals in which extracted tooth sockets were allowed to fill with coagulum and no intervention was done. Group B and C included the animals in which extracted tooth sockets received mucoadhesive paste with and without phenytoin, respectively. Animals of each group were labeled by tags and placed in their respective cages.

All rats were anesthetized with an intraperitoneal injection of 4:1 solution of ketamine (Katafast by Vision Pharmaceuticals Pvt. Ltd., Pakistan)/xylazine (Xylaz by Farvet Laboratories, Netherlands) at a dose of 75 mg/kg of ketamine and 5 mg/kg of xylazine [28] using insulin syringe [29]. The surgical interventions were carried out under sterile conditions. The anesthetized animal was placed on its back on a wooden block. 2% lidocaine without epinephrine (Medicaine® Inj. Huons Co., Ltd.) was diluted up to 0.5% and was used as a local anesthetic according to the standard operating procedure (SOP) set forth by The Institutional Animal Care and Use Committee (IACUC), University of California, San Francisco (UCSF). Buccal Infiltration was given prior to extraction of the tooth [30].

Left first mandibular molar tooth of all animals was extracted [31]. An iris scissor was used as an elevator to elevate the molar tooth of the animals [29]. Group A was labeled as control group with no topical application. 50 mg [32] of each of 1% phenytoin and without phenytoin mucoadhesive pastes were measured on digital analytical balance (ATX 224 UniBloc, Shimadzu Corporation, Japan) and were applied to Group B and Group C, respectively, immediately after the surgery over the extraction wound via sterilized cotton ball [3]. Group B and C received their respective pastes once every 24 h [5], until complete closure of wound was seen [32].

After extraction, all animals were left at room temperature (25 °C) until they recovered from anesthesia [29]. Animals were fed with pasty chow for two days after the surgery and water *ad libitum* [4].

Four animals from each group were placed in a chloroform chamber and euthanized under deep anesthesia on day 7, 14, and 21. Skin and associated tissues were removed. Mandible was washed with saline and resected from the midline (Fig. 1). Mandible was fixed in neutral 10% buffered formalin solution for 48 h at room temperature (25 °C) followed by placement in decalcification solution (5% formic acid) for 2 weeks. Section of the mandibular alveolar ridge area of each rat (containing extracted tooth socket area along with adjacent molar area) was cut in a frontal plane in a bucco-lingual direction [33] and wax blocks were prepared. Blocks were mounted on a rotary microtome (Jung Histocut 820" Leica, USA) and sagittal serial sections of thickness 5 μm [34] were obtained. Slides were prepared and stained using haematoxylin and eosin stains (Sigma-Aldrich, USA). Slides were mounted on optical microscope (Nikon ECLIPSE Ci, Nikon Corporation, Tokyo, Japan). Distal root area of left 1st mandibular molar was recognized and marked mesial to adjacent second molar with the help of microscope camera control unit DS-L3 (Nikon Corporation, Tokyo, Japan) at 4× (Fig. 2).

Four randomly selected sites from the marked area of interest were chosen at 40×. Blood vessels were counted at these sites and their mean was recorded [28]. Percentage of fibroblast cells was calculated by $\frac{Number\ of\ fibroblast\ cells\ per\ field}{Total\ number\ of\ cells\ \text{per}\ field} \times 100$. Mean value of percentage of fibroblast cells was used for analysis.

Percentage of bone trabeculae was measured from the grid overlay method at 10× with the help of microscope camera control unit DS-L3 (Nikon Corporation, Tokyo, Japan) [35] (Fig. 3). Percentage of the bone trabeculae was calculated by $\frac{B}{A} \times 100$. If half or more than half of the box was occupied by bone, then the box was counted and all such boxes were denoted as *B*, whereas, *A* were the total number of boxes occupied within the marked boundary of distal root area.

Statistical analysis

The data was entered and analyzed using IBM® SPSS® Statistics (Statistical Package for Social Sciences) version 27.0. Normality of data was assessed by Shapiro–Wilk test. Evaluation of wound healing in relation to time and treatment factors was done by using two-way ANOVA (analysis of variances). Furthermore, post-hoc Tukey test was performed to assess multiple comparisons between the groups on day 7, 14, and 21 at 5% level of significance. A *p*-value of <0.05 was considered statistically significant.

Examination was done by a calibrated histopathologist. The inter-examiner reliability was determined using Intraclass correlation coefficient (0.83) which means a good agreement between the observers.

Results

Blood vessel count

The blood vessel count, on day 7, for Group A, B, and C were 11.62 ± 0.85, 12.62 ± 0.85, and 12.00 ± 1.58, respectively (Tab. I). Day 14 showed that there was increase in blood vessel count among the groups as compared to day 7, with values of 18.62 ± 1.35, 22.25 ± 3.30, and 19.06 ± 1.19 in Group A, B, and C, respectively (Tab. II). On day 21, a decrease in blood vessel count was observed among the groups in comparison to day 14 (Fig. 4). Furthermore, the blood vessel count, on day 21 was almost similar in all the groups (Tab. III). No significant difference was observed among the groups on day 7, 14, and 21 (Tabs. I–III). Figure 4 depicts the graphical representation of the average number of blood vessel count among the groups on day 7, 14, and 21.

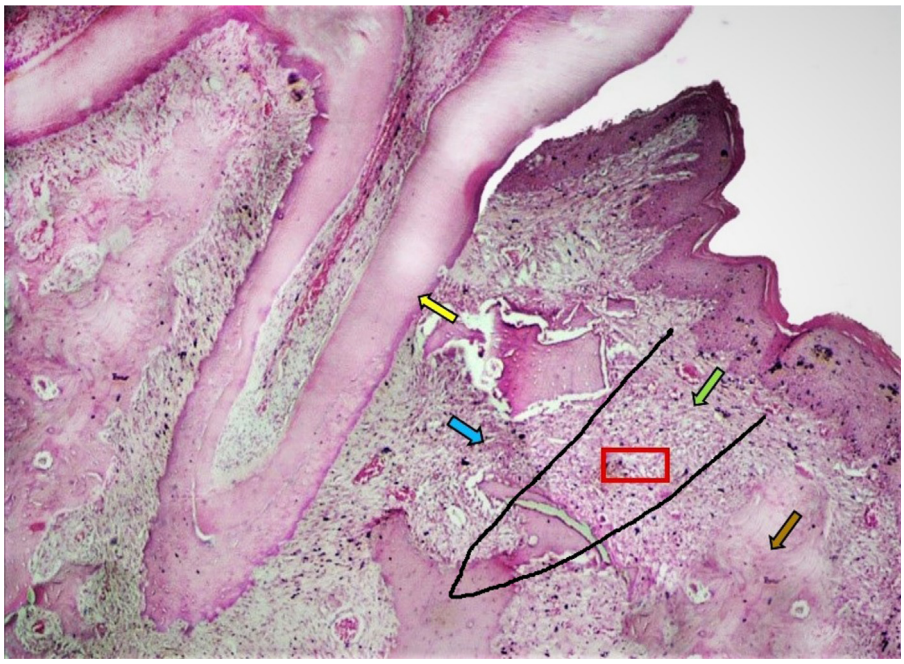

Fig. 2. Photomicrograph of extracted tooth socket of Group A (control), 7 days after extraction, H and E stain, 4X. The image depicts the mesial root of 2nd mandibular molar (yellow arrow). Adjacent to it is the distal root area of extraction socket of 1st mandibular molar (blue arrow). Black line shows the distal root morphology of 1st mandibular molar. Alveolar socket is also evident (green arrow) with adjacent interradicular bone (brown arrow). Red rectangular area highlights part of the area of extraction socket.

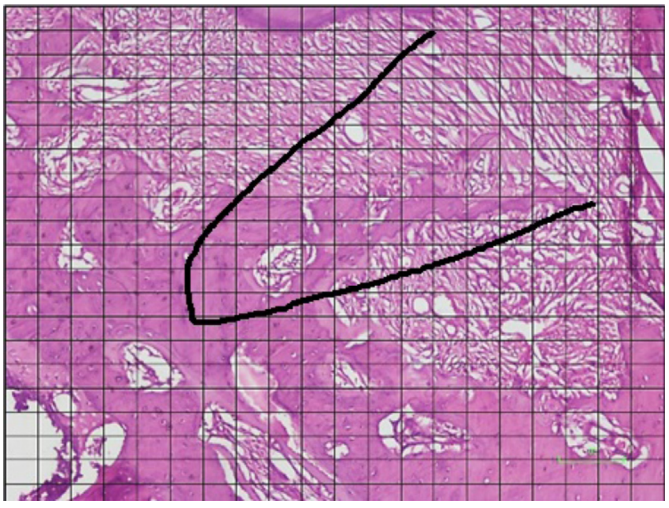

Fig. 3. Measuring percentage of bone trabeculae at 10X.

Percentage of fibroblast cells

According to this study, Group B (73.79 ± 1.80) showed a statistically significant increase in percentage of fibroblasts on day 7, as compared to Group A (50.19 ± 2.54; p-value < 0.001) and C (53.31 ± 1.18; p-value < 0.001). Meanwhile, difference between Group A and C was not significant (p-value = 0.10) (Tab. I). On the other hand, day 14 showed no significant difference in the percentage of fibroblast among the groups (Tab. II). However, on day 21, Group B (35.37 ± 1.57) showed a significant decrease in the percentage of fibroblasts in comparison to Group A (39.84 ± 1.97; p-value = 0.001) and C (43.22 ± 1.33; p-value < 0.001). No significant difference was observed between Group A and C (p-value = 0.091) (Tab. III). Bar graph showing the change in percentage of fibroblast cells among the three groups on day 7, 14, and 21 is illustrated in Figure 5.

Table I. Descriptive analysis showing mean, standard deviation (SD) and Post Hoc Tukey test of histological variables of Group A, B and C at day 7.

Variables	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Post Hoc Tukey test		
Blood vessel count	11.62 ± 0.85	12.62 ± 0.85	12.00 ± 1.58	A	B	0.46
					C	0.89
				B	A	0.46
					C	0.73
				C	A	0.89
					B	0.73
Percentage of fibroblasts	50.19 ± 2.54	73.79 ± 1.80	53.31 ± 1.18	A	B	<0.001*
					C	0.10
				B	A	<0.001*
					C	<0.001*
				C	A	0.10
					B	<0.001*
Percentage of bone trabeculae	36.23 ± 1.66	44.12 ± 1.45	33.98 ± 1.19	A	B	<0.001*
					C	0.126
				B	A	<0.001*
					C	<0.001*
				C	A	0.126
					B	<0.001*

*$p < 0.05$.

Table II. Descriptive analysis showing mean, standard deviation (SD) and post hoc Tukey test of histological variables of Group A, B and C at day 14.

Variables	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Post Hoc Tukey test		
Blood vessel count	18.00 ± 1.35	22.25 ± 3.30	19.06 ± 1.19	A	B	0.052
					C	0.774
				B	A	0.052
					C	0.151
				C	A	0.774
					B	0.151
Percentage of fibroblasts	45.72 ± 3.01	44.58 ± 2.04	46.46 ± 1.77	A	B	0.779
					C	0.895
				B	A	0.779
					C	0.518
				C	A	0.895
					B	0.518
Percentage of bone trabeculae	48.43 ± 1.73	53.77 ± 1.45	46.11 ± 1.69	A	B	0.003*
					C	0.164
				B	A	0.003*
					C	<0.001*
				C	A	0.164
					B	<0.001*

*$p < 0.05$.

Table III. Descriptive analysis showing mean, standard deviation (SD) and Post Hoc Tukey test of histological variables of Group A, B and C at day 21.

Variables	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Post Hoc Tukey test		
Blood vessel count	12.87 ± 1.25	12.62 ± 1.25	12.37 ± 0.85	A	B	0.948
					C	0.811
				B	A	0.948
					C	0.948
				C	A	0.811
					B	0.948
Percentage of fibroblasts	39.84 ± 1.97	35.37 ± 1.57	43.22 ± 1.33	A	B	0.001*
					C	0.091
				B	A	0.001*
					C	<0.001*
				C	A	0.091
					B	<0.001*
Percentage of bone trabeculae	60.09 ± 1.40	67.03 ± 1.51	57.65 ± 1.72	A	B	<0.001*
					C	0.120
				B	A	<0.001*
					C	<0.001*
				C	A	0.120
					B	<0.001*

*$p < 0.05$.

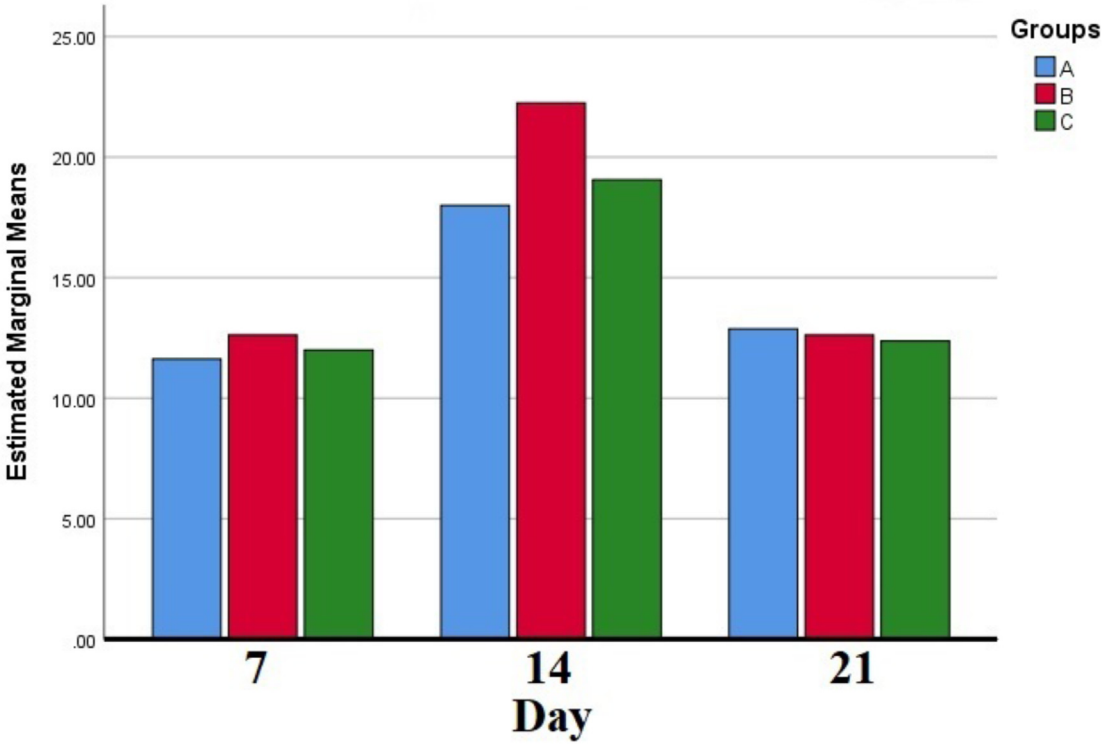

Fig. 4. Estimated marginal means of blood vessel count on day 7, 14, and 21.

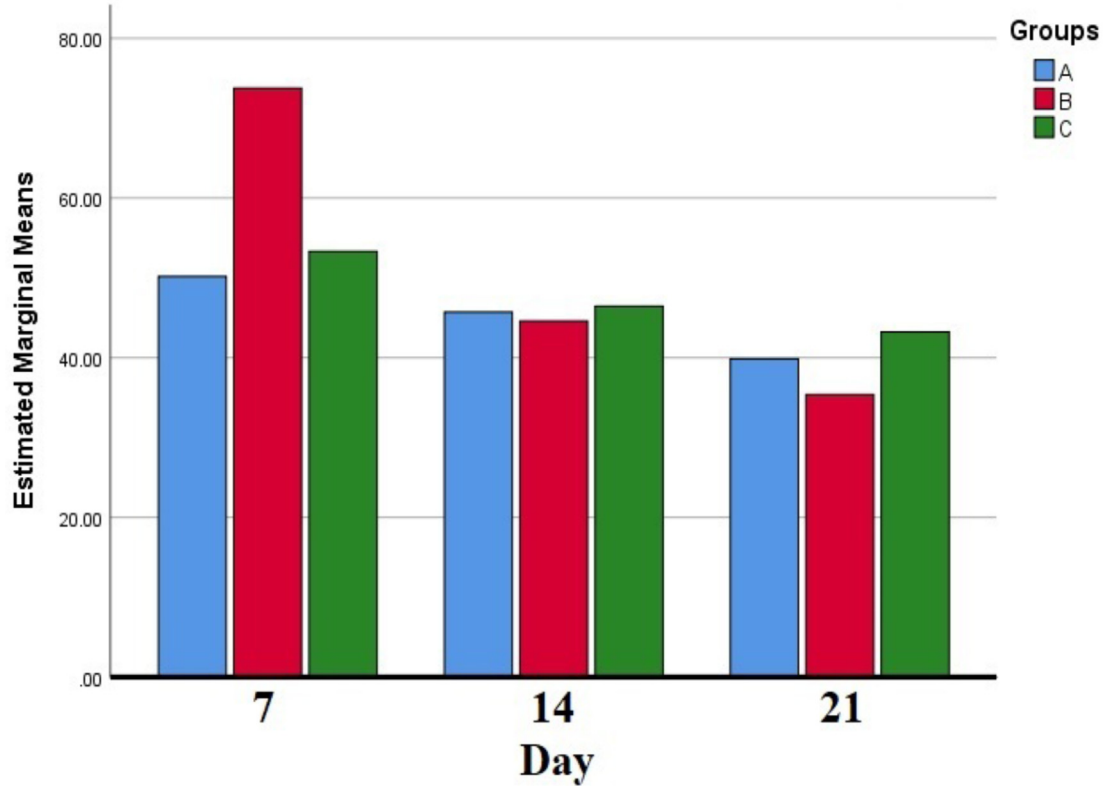

Fig. 5. Estimated marginal means of percentage of fibroblast cells on day 7, 14, and 21.

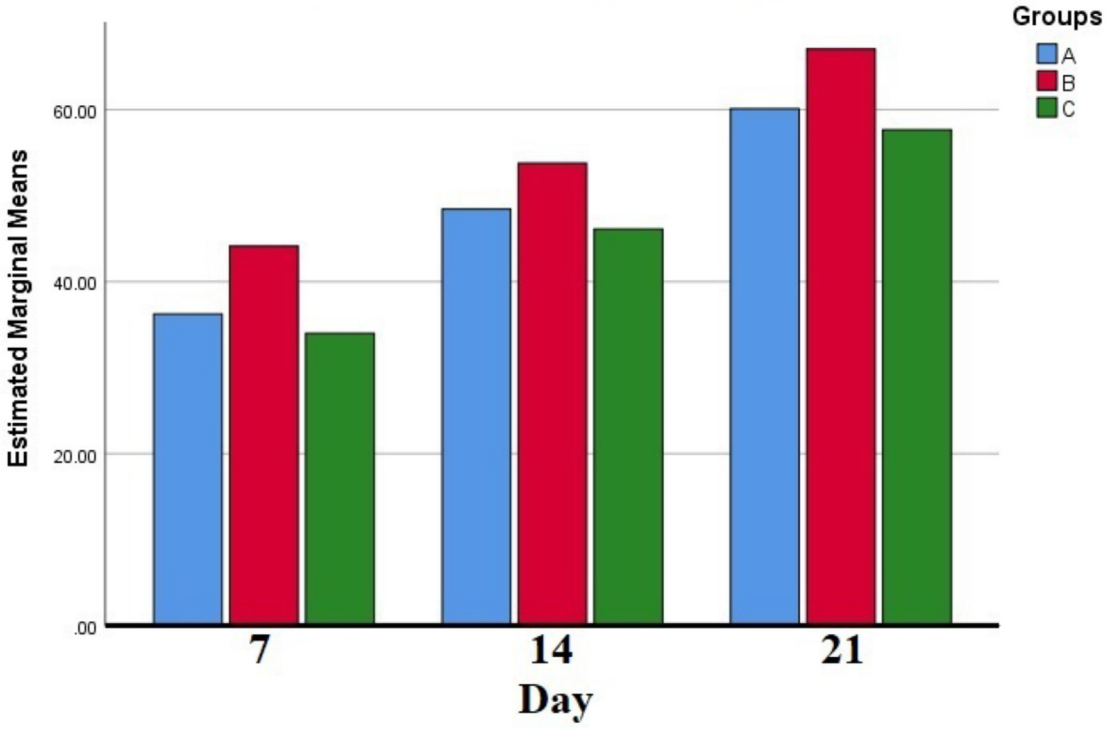

Fig. 6. Estimated marginal means of percentage of bone trabeculae on day 7, 14, and 21.

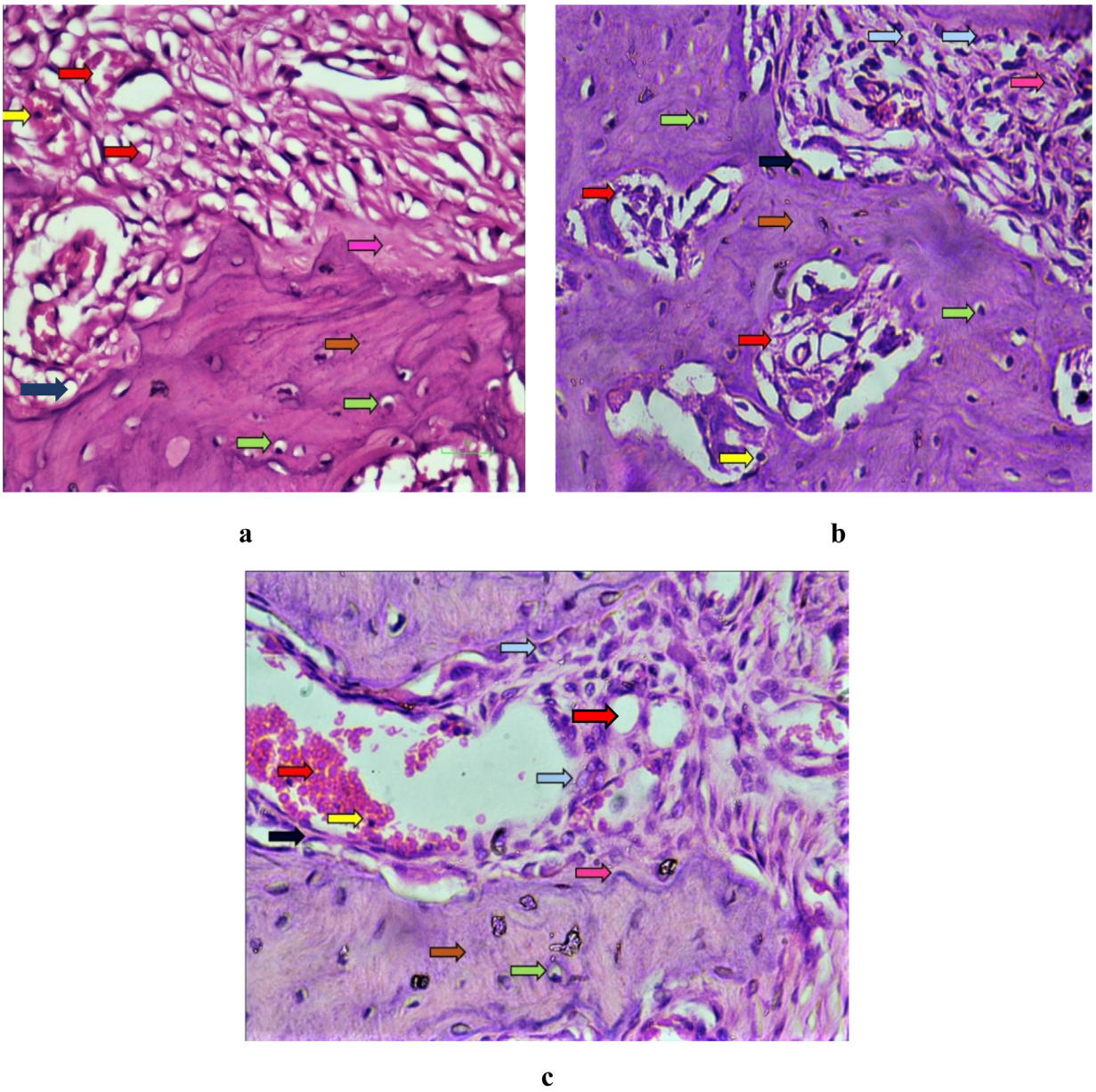

Fig. 7. Photomicrograph of Group A (control) (a), B (mucoadhesive paste with phenytoin) (b), and C (mucoadhesive paste without phenytoin) (c), 14 days after extraction, H and E stain, 40×. Blood vessels (red arrows) are seen with inflammatory cells (yellow arrow). Fibroblasts are mostly arranged in a symmetrical manner. Ground substance (pink arrow) is evident with bone tissue. Mineralized bone matrix (brown arrow) is seen having bone forming cells along the borders (dark blue arrows). Osteocytes are also evident within the bone tissue (green arrow).

Percentage of bone trabeculae

As shown in Figure 6, bone formation was evident in all the groups. Group B (44.12 ± 1.45) showed a statistically significant increase in percentage of bone trabeculae in comparison to Group A (36.23 ± 1.66; p-value < 0.001) and C (33.98 ± 1.19; p-value < 0.001) on day 7 (Tab. I).

A significant increase in percentage of bone trabeculae was observed on day 14 in all the groups. Furthermore, Group B (53.77 ± 1.45) showed a significant increase in percentage of bone trabeculae in comparison to Group A (48.43 ± 1.73; p-value = 0.003) and C (46.11 ± 1.69; p-value < 0.001). This increase in percentage of bone trabeculae among the groups was also observed on day 21. Post-hoc Tukey analysis showed that Group B (67.03 ± 1.51) has a significant increase in percentage of bone trabeculae in comparison to Group A (60.09 ± 1.40; p-value < 0.001) and C (57.65 ± 1.72; p-value < 0.001). No significant difference was observed between Group A and C on day 7, 14, and 21 (Tabs. I–III).

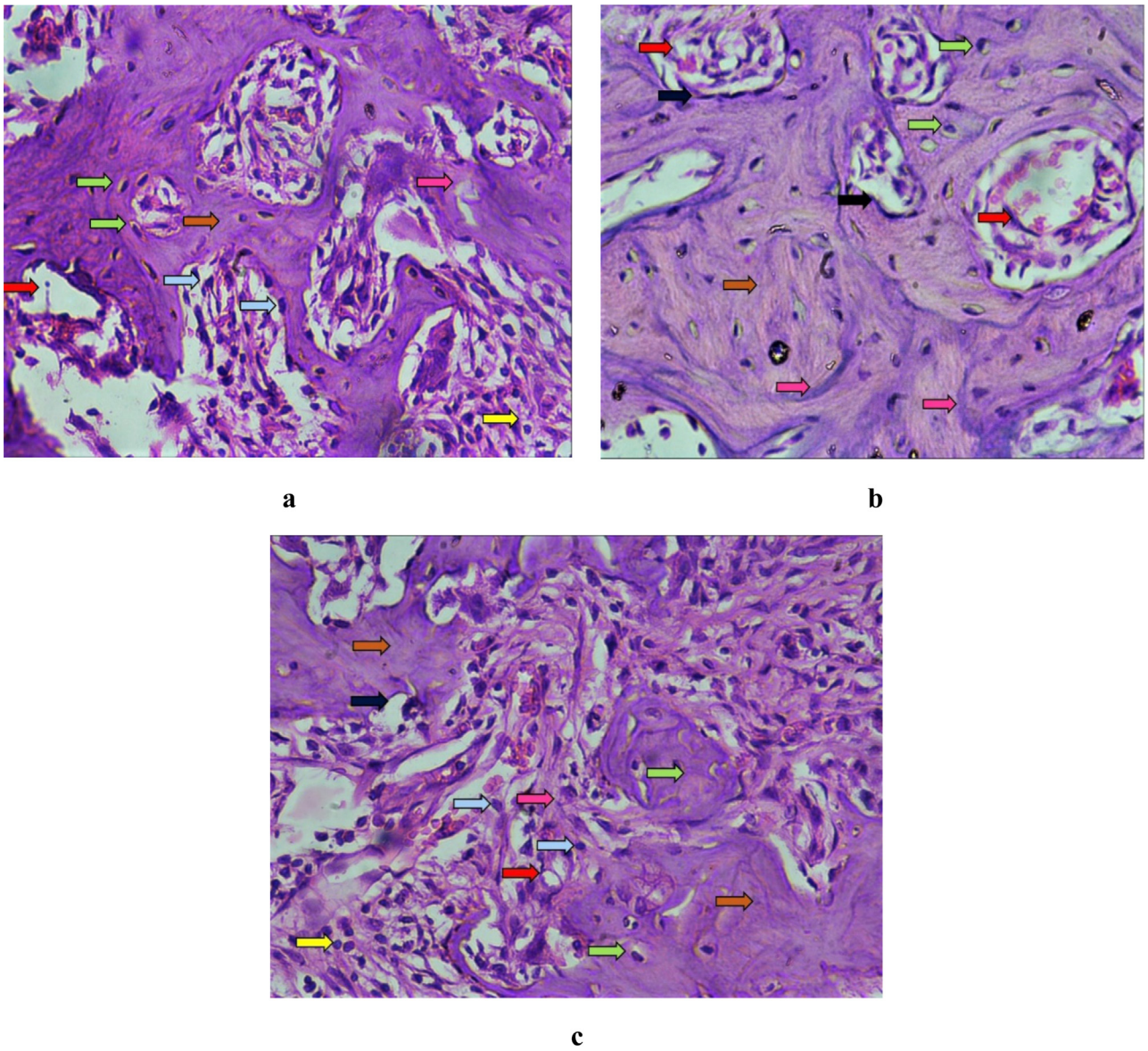

Fig. 8. Photomicrograph of Group A (control) (a), B (mucoadhesive paste with phenytoin) (b), and C (mucoadhesive paste without phenytoin) (c), 21 days after extraction, H and E stain, 40×. These images depict new bone formation. Blood vessels (red arrow), inflammatory cells (yellow arrow), osteocytes (green arrow) are seen. Well-developed haversian system can been seen in (b). Bone lining cells (dark blue arrow) marginate the bone matrix. Mineralized bone matrix (brown arrow) is seen having bone forming cells (light blue arrow) in proximity to its mineralization front (pink arrow).

Discussion

The most significant finding of the present work was that phenytoin mucoadhesive paste accelerated the healing process of extracted tooth socket of albino Wistar rats by promoting fibroblast proliferation and early osteogenesis. In this study, left mandibular molar was selected to access wound healing of extraction socket. The reason for the selection of left mandibular molar was the ease for a right-hand operator to extract left first mandibular molar as compared to the right first mandibular molar. Other reasons included the ease of daily application of study material to the mandibular extraction socket of rat as compared to maxillary socket and increase incidence of complications like delayed wound healing/dry socket associated with mandibular molars [36].

The quantitative analysis of percentage of fibroblasts and formation of bone trabeculae exhibited statistically significant results, whereas, difference in blood vessel count among the groups was not significant.

Neoangiogenesis is a property associated with phenytoin treated healing wounds. On day 7 and 14, this study showed an increased number of blood vessels (neoangiogenesis) in Group B as compared to Group A and C (Tabs. I and II). However, the difference was not significant. Abundance of blood vessels were found in the apical third followed by middle third in all the groups.

An interesting fact regarding the number of blood vessels was noted in this study. Histological analysis showed that as the healing progressed from day 7 to 14, the number of blood vessels increased in all the samples, however, blood vessel count was seen to be decreased on day 21. This finding has also been described in other studies which states that blood vessels decrease in number in terminal phases of a healing wound [28,37] as collagen begins to accumulate in the granulation tissue [38], while others proposed that the metabolic demands in the repaired tissue no longer necessitates such an extensive vasculature [37]. Hence, most of the blood vessels degenerate via apoptosis [39].

The next phase of healing that follows is the proliferative phase. In this study, the percentage of fibroblasts between Group A and C (that is, on day 7, 14, and 21) was statistically not significant (Tabs. I–III), whereas, Group B showed a statistically increased percentage of fibroblasts on day 7 as compared to Group A and C. This finding agrees with the work published by Hagh and co-workers in which there is increase in fibroblast count in group following phenytoin gel application as compared to control [28].

Phenytoin effect on fibroblast proliferation and collagenase activity has been published [19,40,41]. Increase in fibroblast count in phenytoin treated group owes to the increase levels of TGF-β [16] along with increase in gene expression of PDGF (platelet derived growth factor) and TGF-β receptors [42], which results in upregulation of their release from macrophages [28]. Another effect of phenytoin is that it acts on basal epithelial cells to produce connective tissue growth factor (CTGF) [43]. These growth factors not only enhance the proliferative activity [42], but also increases the synthetic and migratory activity of fibroblasts [18]. Phenytoin is also advocated for epithelial to mesenchymal transition in which the basal cells of epithelia lose their polarity and transforms into fibroblasts along with increase in their mitotic activity. Another proposed mechanism is that phenytoin increases fibroblast count by decreasing their apoptosis cycle [42]. Day 14 showed that the difference in percentage of fibroblasts between the groups was not significant (Tab. II). Day 21 showed that the percentage of fibroblast decreased in all the groups as compared to day 14 (Tabs. II, III and Fig. 5). This finding is in accordance with the work of Hagh and colleagues in which they found out that the fibroblast content reaches up to a certain limit and then declines as healing ensues [28]. Percentage of fibroblasts in Group B was statistically decreased on day 21 in comparison to Group A and C (Tab. III) because the area that was previously occupied by fibroblasts and ground substance (day 7 and 14) is now greatly occupied by bone matrix and bone cells. Proposed mechanism is that the decrease in the number of fibroblasts at the extraction site is due to the differentiation of fibroblasts into osteoblasts, which formed new bone [34].

According to Simpson's study, the formation of new bone at the site of extraction socket starts by day 5 after extraction [44]. Percentage of new bone formation was also evaluated in this study. Post-hoc analysis on day 7, 14, and 21 showed that the percentage of bone trabeculae in Group A and C was statistically not significant

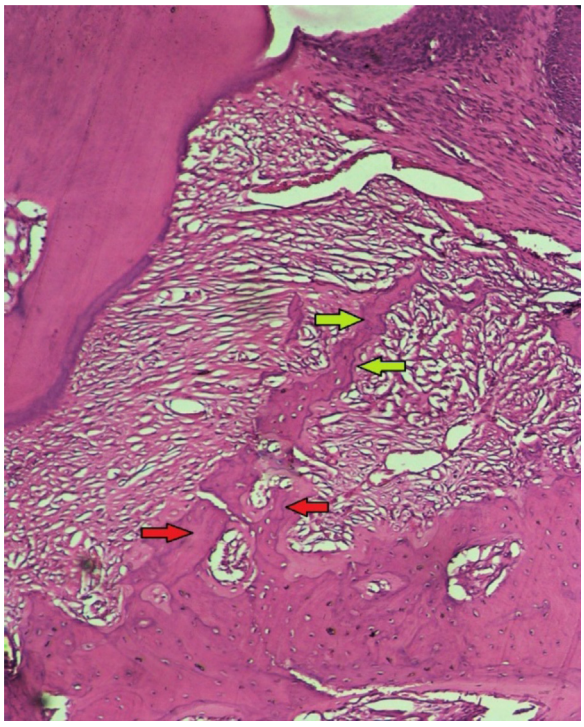

Fig. 9. Photomicrograph of histological section of extraction socket at 10×; Group B, day 14, showing the pattern of bone formation. Bone formation starts from apical region (red arrows) and advances towards middle third (green arrows).

(Tabs. I–III), whereas, Group B had a significant increased difference in percentage of bone trabeculae as compared to Group A and C on all the days (Tabs. I–III, Figs. 6). Fresh bone was observed in all the samples at day 7 in the apical region.

Day 14 showed that almost all the apical part was occupied by bone. Bone matrix, bone cells along with developing bone trabeculae were seen in all the samples (Fig. 7). Increased percentage of bone trabeculae was observed on day 21 in all the samples (Fig. 8). Day 21 also showed that almost all the socket was occupied by bone cells or bone.

To summarize the pattern of bone formation concluded from this study, it can be stated that bone formation starts from the apical region and progresses to middle third (Fig. 9). Same observation was also reported in other studies [34,44]. Bone formation was seen to be more in phenytoin treated group in this study. This is because phenytoin increases the release of several growth factors such as PDGF, TGF-β, VEGF, and FGF (fibroblast growth factor) [38] after extraction. TGF-β, FGF, and EGF (epidermal growth factor) are involved in activation and recruitment of osteoblasts. These factors have been evidenced in the early and intermediate stages of bone formation in extraction socket [45].

The effect of phenytoin in a healing bone model has also been studied by other researchers and they advocated the positive effect of phenytoin on bone healing [15,46,47], which is in accordance with the findings of this study.

Conclusion

In this study, histological features were compared among Group A (control), B (mucoadhesive paste with phenytoin), and C (mucoadhesive paste without phenytoin). Phenytoin not only increased fibroblasts count but also promoted bone formation. This supports that phenytoin has an effective role in promoting wound healing and early osteogenesis.

Limitation of the study

1% phenytoin mucoadhesive paste was used in a rat model in this study. This regime can not be applied directly for clinical use since the time of healing of a rat extraction socket and human is different.

Future aspects

Currently, the use of topical phenytoin is not approved by USA Food and Drug Administration (FDA). However, FDA has approved the originally systemic products for topical treatment of wounds including anesthetics, antibiotics, and growth factors. For such purposes, an "off-label prescription medication" term is used according to which phenytoin use (in humans) as a topical agent is within the guidelines set forth by FDA. Furthermore, phenytoin cream is available in France for topical use. In 1984, a paper was published by the FDA office of Orphan Products Development, which listed phenytoin as a potentially useful agent in wound healing. Phenytoin is cheap and readily available drug. Analysis on the molecular factors involved in the mechanism of action of phenytoin should be carried out. Furthermore, different concentrations of 0.5%, 2%, and 3% phenytoin mucoadhesive paste should be investigated to evaluate the outcomes of different concentrations on wound healing of extraction sockets.

Conflict of Interest

None declared.

Informed consent

Informed consent was not applicable to this study as it was done on rats.

Ethical approval

The research project was approved by the Ethical Committee of Postgraduate Medical Institute, Lahore, Pakistan (PGMI /AMC/ LGH / Research No. 00-41-S-2017 / Dated/ 21-04-2017) and the Advanced Studies and Research Board of the University of Health Sciences, Lahore, Pakistan (No: UHS/Education/126-18/598, dated 06-02-2018).

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