

# Evaluating the effect of laser irradiation on bone regeneration in midpalatal suture concurrent to rapid palatal expansion in rats

Fariborz Amini, Maryam Pirmoradian Najaf Abadi<sup>1</sup> and Mobina Mollaei

## ABSTRACT

**Background:** Rapid palatal expansion is one of the most important orthopedic treatments that correct the dental and palatal constriction. Stability of the changes partly depend on the rapidity of new bone formation in affected sutures after expansion. The purpose of this study was to investigate the effect of laser irradiation on the healing of midpalatal suture concurrent to the expansion of midpalatal suture in rats.

**Materials and Methods:** A total of 78 male Sprague rats in seven groups were evaluated: A control group of six rats without any treatments and three experimental groups of 24 which underwent palatal expansion for different time periods (7, 14, and 30 days), and each divided into two groups of with and without laser irradiation. Laser therapy was done by gallium-aluminum-arsenide diode laser with 810 nm wavelength and 4 J/cm<sup>2</sup> irradiation in days 0, 2, 4, 6, 8, 10, 12, 14 in 4 points (1 labial and 3 palatal points). After sacrificing, the sections were evaluated by histomorphometric and quantitative analysis and results were statistically investigated by independent samples *t*-test.

**Results:** The results in 7 days, 14 days, and 30 days show that laser therapy can increase the rate of osteogenesis in palatal suture during rapid palatal expansion but the differences in 7 days groups were not significant ( $P = 0.117$ ) while in 14 days groups ( $P = 0.032$ ) and 30 days groups were significant ( $P = 0.001$ ). Most of effectiveness of low-power laser was seen between 14 and 30 days while the laser therapy was stopped.

**Conclusion:** These findings suggest that low-level laser irradiation can increase and accelerate bone regeneration in the midpalatal suture after rapid palatal expansion, hence, reduce retention time.

**Key words:** Bone regeneration, low-power laser irradiation, maxillary expansion, rapid palatal expansion, rat

## INTRODUCTION

Rapid maxillary expansion is one of the treatment options in the correction of the constriction of maxillary dental arch and jaw. Maxillary constriction is a part of many deformities such

as posterior crossbites, class II and III malocclusion.<sup>[1,2]</sup> One of the reasons for relapse consequence to use of maxillary rapid palatal expander (RPE) is a lack of rapid and adequate bone regeneration in the midpalatal suture.<sup>[3]</sup> Long-time retention after expansion will significantly decrease the rate of relapse through bone regeneration in palatal midsuture.<sup>[4,5]</sup> On the other hand, long-term use of banded or bonded RPE as retainers can cause some complications such as enamel hypocalcification, gingival irritation and dental carious. Improved bone regeneration in

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the midpalatal suture decreases the retention time, and its complications hence improves treatment stability. Maxillary expansion causes hyperemia, osteoblastic activity, primary bone regeneration, and calcium removal.<sup>[6-9]</sup>

Since 1971, studies have evaluated the effect of laser on bone regeneration in clinical conditions or cell cultures.<sup>[2,4,5,9-15]</sup> Lasers can be used in biopsy sampling, eliminating oral lesions (aphthous ulcers, herpetic lesions), tuberosity reduction, coagulation, treating temporomandibular joint lesions, instrument sterilization, gingivectomies, etc.<sup>[2,4,5,7-19]</sup>

Healing is one of the implications of low-power laser therapy. Regeneration is a complicated process and laser might accelerate it.<sup>[2,4,5,8-14,20-22]</sup> In a study, Saito and Shimizu investigated the effect of gallium-aluminum-arsenide (Ga-Al-As) low-power laser exposure on bone regeneration in palatal midsuture during the maxillary expansion of rats.<sup>[4]</sup> Results of histophotometric investigations showed that compared with the control group, bone regeneration increased approximately 1.2–1.4 times in 7 days in the experimental group. In addition, laser irradiation was more effective in the first 3 days. These results confirm the positive effect of laser therapy on bone regeneration in the midpalatal suture.<sup>[4]</sup> Blaya *et al.* studied effects of laser irradiation on the bone regeneration and showed increased width and depth of newly bone.<sup>[20]</sup> Agaiby *et al.* showed that areas receiving laser therapy experienced less pain and more effective dental movement.<sup>[23]</sup> Kawasaki and Shimizu reported better tooth movement, greater bone regeneration and cellular proliferation, and a greater number of osteoclasts in areas exposed to the laser.<sup>[24]</sup> In a study Ozawa *et al.* showed that laser exposure in early stages of culture, significantly stimulates cell proliferation, alkaline phosphatase activity, and appearance of osteocalcin gene.<sup>[21]</sup> Furthermore, laser exposure in early stages of culture caused a significant increase of bone nodules in 21<sup>st</sup> day. In other words, it caused both cell proliferation and cell differentiation.<sup>[21]</sup>

This study was conducted to evaluate the influence of Ga-Al-As low-power laser exposure on bone regeneration in palatal midsuture during palatal expansion in rats.

## MATERIALS AND METHODS

A total of 78 Sprague male rats, each weighted around 200 g were randomly selected. The study was performed on six experimental groups of 12 rats, which underwent palatal expansion and one control group of six rats, which have not gone under any intervention. The animals were quarantined for 1 week. Rats' weight was monitored, and rats experiencing weight loss were kept in separate cages until reaching the normal weight of control group.

Rats were randomly divided into three experimental groups of 24 rats and one control group of 6 rats:

- Control group with no expansion or laser therapy
- Experimental group under treatment for 7 days

- Experimental group under treatment for 14 days
- Experimental group under treatment for 30 days.

Each one of the above experimental groups was divided randomly into two groups of expansion without laser irradiation “a” and expansion with laser irradiation for maximum 14 days “b”.

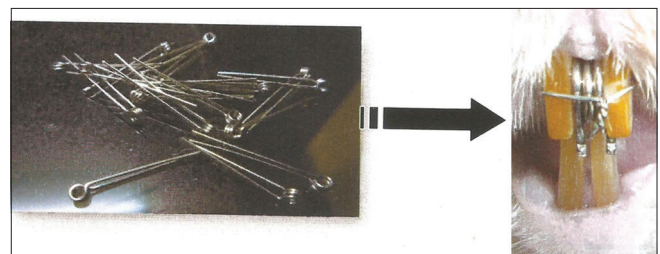
Before placing orthodontic appliances, all experimental rats received 10% animal Ketamine and 2% xylocaine intramuscular injection as deep sedation.<sup>[25]</sup> For expansion first an osteom was used to open a small space between central incisors, then a coil made of 1.5 mm stainless steel wire was used to open the midpalatal suture [Figure 1].<sup>[4]</sup> Coil was slowly pushed between central incisors. To stabilize the ring, a hole was drilled using a 35,000-rpm hand-piece in the distal portion of both centrals along the gingival papillae [Figure 2]. The ring, was stabilized using 0.5 mm round brass wire. In the days 0, 2, 4, 6, 8, 10, 12, and 14, rats were irradiated by laser beam using a Ga-Al-As laser with wavelength of 810 and energy density of 4 J/cm<sup>2</sup> (Photo Laser, DMC, Brazil) in 4 points by placing the laser tip vertically over the mucosa as it has been described by Saito and Shimizu<sup>[4]</sup> and Blaya *et al.*<sup>[20]</sup> [Figure 3].

- One point with Buccal: 0.5 mm apically to the edge of bone crest
- Three points in palatal:
  - a. 1 mm apically to the edge of bone crest
  - b. 2 mm apically to the edge of bone crest
  - c. 3 mm apically to the edge of the bone crest.

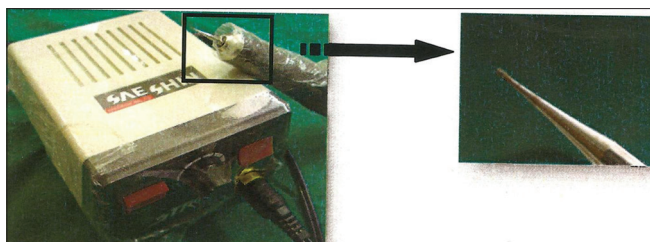
After expansion and irradiation in 3 times intervals (7, 14, and 30 days), exposed (n=12) and unexposed (n=12) rats were anesthetized by injection of sodium pentobarbital and stored in 10% formalin with volume ratio of 1:20 and then sacrificed<sup>[26]</sup> [Figure 4]. For histological evaluation, optical microscopy and computer software (Photoshop Cs3, Adobe, USA) were used [Figure 5]. Findings were analyzed using independent samples *t*-test. Findings were considered significant when  $P \leq 5\%$  ( $P < 0.05$ ).

## RESULTS

Distribution of weight mean changes in irradiated groups and nonirradiated groups with their living days are given in Table 1.



**Figure 1:** Stainless steel ring 1.5 mm thick, made from 0.5 mm wires, and having two extended bars for ease of use



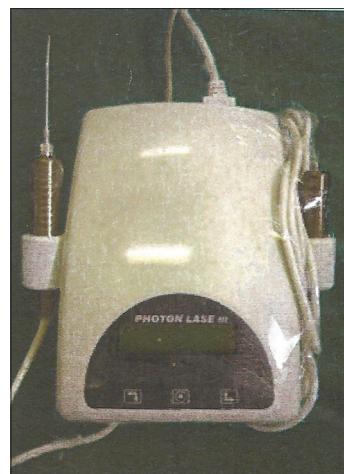
**Figure 2:** The straight handpiece (maximum rpm: 35,000) with a round carbide ¼ bur



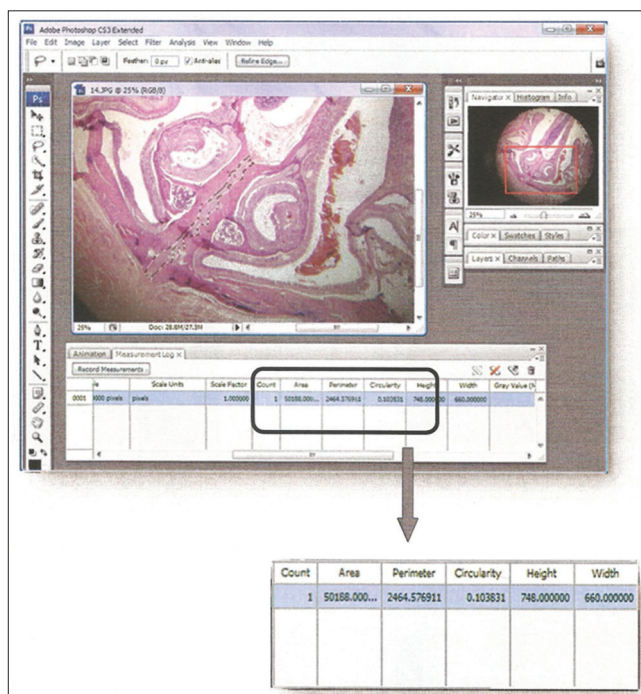
**Figure 4:** Rat maxillary bone showing laser spots. It was stored in 10% formalin with volume ratio of 1:20

During 7 days, weight changes of rats in group “2b” was significant compared with rats in group “2a”. Accordingly, weight reduced in all groups during first 2–4 days. This decrease occurred after appliance placement and using the first dose of anesthesia, but weight loss in all laser-treated groups “b” was significantly less than weight loss of no-laser experimental groups “a.” While most rats in experimental groups “b” showed weight loss only for 2 days, rats in experimental groups with no laser therapy “a” continued losing weight until 4<sup>th</sup> day, and many of them were not able to recover their initial weight after 7 days. Rats in experimental groups “b” started gaining weight at a rate equal to control, in a way that after 7 days, most of them could recover their weight loss in the first 2 days. Weight changes during this timescale were also significant among groups. Weight gain in groups continued during days 6, 8, 10, 12 and 14 until the group without laser therapy “a” completely recovered in the 9<sup>th</sup> day. Weight changes of group “3b” in comparison with group “3a” were also significant during 2<sup>nd</sup> to 6<sup>th</sup> days. During 10<sup>th</sup> to 14<sup>th</sup> days, the mean of weight gain in laser groups “b” was more, but we did not observe any significant difference between two groups. Weight gain process in groups of 30 days continued until the 30<sup>th</sup> day. Weight increases of both groups were similar in 14<sup>th</sup> day. After 14 days, weight gain in experimental groups “b” and “a” were similar.

Changes in mean weight (g/day) of experimental groups are compared in Diagram 1.



**Figure 3:** The gallium-aluminum-arsenide low-power laser device



**Figure 5:** Surface areas were measured on digital images using Adobe Photoshop Cs3

Quantitative bone changes [Table 2]:

### Bone Regeneration in 7<sup>th</sup> Day

The extent of bone regeneration in group “2a” was  $37.6 \pm 1.7 \times 10^{-3}$  inches, while it was  $40.2 \pm 3.15 \times 10^{-3}$  inches in group “2b.” Difference between groups “2a” and “2b” was not significant ( $P > 0.05$ ) [Figure 6 and Diagram 2]

### Bone Regeneration in 14<sup>th</sup> Day

Mean bone regeneration in groups “3b” and “3a” were  $41.7 \pm 2.8 \times 10^{-3}$  and  $38.8 \pm 1.85 \times 10^{-3}$  inches, respectively. Statistically significant differences between two exposed and unexposed groups were observed ( $P = 0.032$ ) [Figure 7 and Diagram 3].

**Table 1: Distribution of mean weight changes in exposed (a) and unexposed (b) groups**

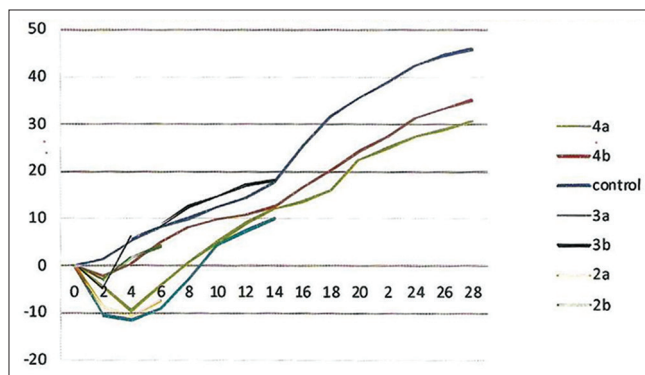
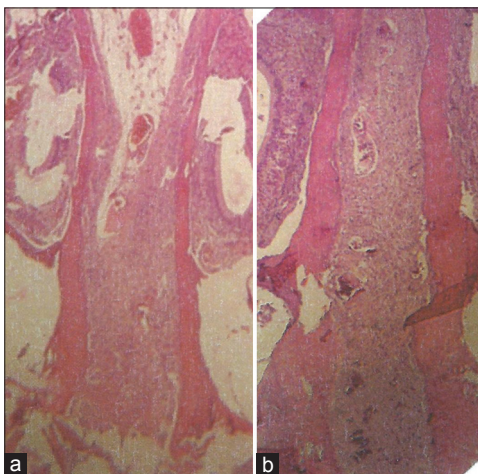
Group	Number of animals	Initial weight mean (g)	Weight gain mean (g/day)	SD (g)	Minimum weight gain (g)	Maximum weight gain (g)
Intact	6	175.80	1.68	7.79744	40.7182	60.0818
2a	12	189.58	-0.5457	9.77474	-11.7106	0.7106
2b	12	180.16	0.7738	6.302336	1.41123	9.4210
3a	12	180.3	0.6722	11.66548	2.6714	17.4952
3b	12	175.3	0.7	9.79912	4.0239	16.4761
4a	12	180.08	0.8722	1.06673	18.4998	33.8335
4b	12	182.66	1.1274	16.55567	23.8194	43.8283

SD – Standard deviation

**Table 2: The mean of bone regeneration in study groups**

Group name	Number of animals	Mean of bone regeneration (mm×10 <sup>-3</sup> inches)	Minimum bone regeneration (mm×10 <sup>-3</sup> inches)	Maximum bone regeneration (mm×10 <sup>-3</sup> inches)	P
2a	12	37.6±1.7	35.9	39.3	0.117
2b	12	40.2±3.15	37.1	43.4	
3a	12	38.8±1.85	37.0	40.7	0.032*
3b	12	41.7±2.8	37.9	43.5	
4a	12	38.4±2.5	35.9	40.9	0.001**
4b	12	43.6±1.65	41.9	45.2	

\*P&lt;0.05; \*\*P&lt;0.01

**Diagram 1: Mean weight changes of control group and study groups (g/day)****Figure 6: Histological view of midpalatal suture, 7 days after expansion (a) without laser therapy and (b) with laser therapy**

### Bone Regeneration in 30<sup>th</sup> Day

However, radiation were stopped in 15 days but after

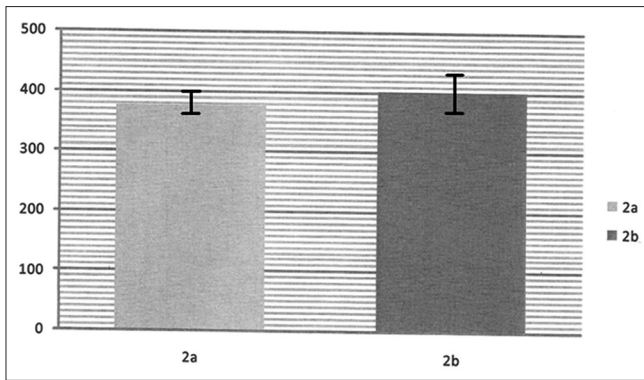
30 days bone regeneration in unexposed group (“4a”) was  $37.6 \pm 1.7 \times 10^{-3}$  inches, while it was  $40.2 \pm 3.15 \times 10^{-3}$  inches in exposed group (“4b”). This difference was statistically significant ( $P = 0.001$ ) [Figure 8 and Diagram 4].

In each day of 1<sup>st</sup> week after expansion, mean of bone regeneration for group “2b” was  $5.7 \times 10^{-3}$  inches while this amount was  $5.3 \times 10^{-3}$  inches for group “2a”. Mean of bone regeneration during next 7 days in group without laser therapy was  $1.2 \times 10^{-3}$  inches and was  $0.4 \times 10^{-3}$  inches in the laser group. It means that the mean of bone regeneration in each day of 2<sup>nd</sup> week for group “3a” was  $0.1 \times 10^{-3}$  inches and for group “3b” was  $0.06 \times 10^{-3}$  inches that showed a drastic decrease. In 3<sup>rd</sup> and 4<sup>th</sup> week, bone regeneration decreased for  $0.4 \times 10^{-3}$  inches in rats not treated with the laser, while it increased for about  $2.8 \times 10^{-3}$  inches in rats, which received laser irradiation. According to this statistics, the greatest amount of bone regeneration in first 7 days was in group 2b while the greatest effect on bone regeneration in groups with laser therapy against groups without laser therapy were observed during second 15 days.

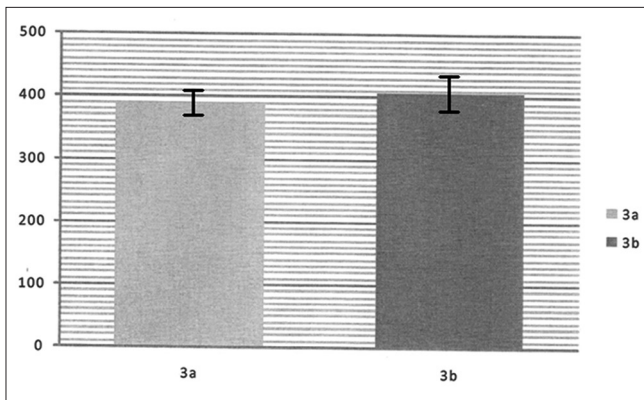
## DISCUSSION

This study has evaluated changes in different timescales using the histomorphometric method. Furthermore, in this study, samples were standardized. Therefore, it has provided more realistic evaluation of regenerated bone on different timescales.

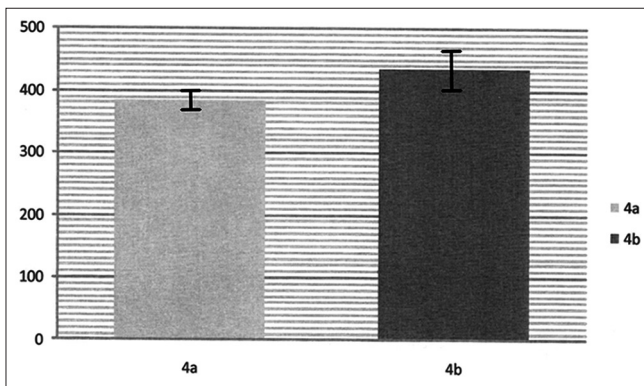
Weight loss of rats in initial days after placing appliance is probably due to pain and trauma of rapid maxillary expansion. According to previous studies, laser can reduce



**Diagram 2:** Mean bone regeneration (in 0.001 inch) after 7 days. The difference was not significant



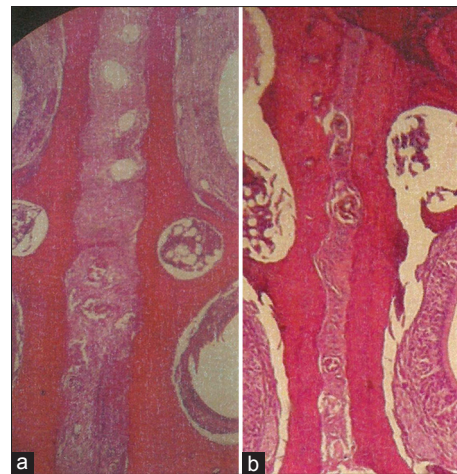
**Diagram 3:** Mean bone regeneration (in 0.001 inch) after 14 days. The difference was significant



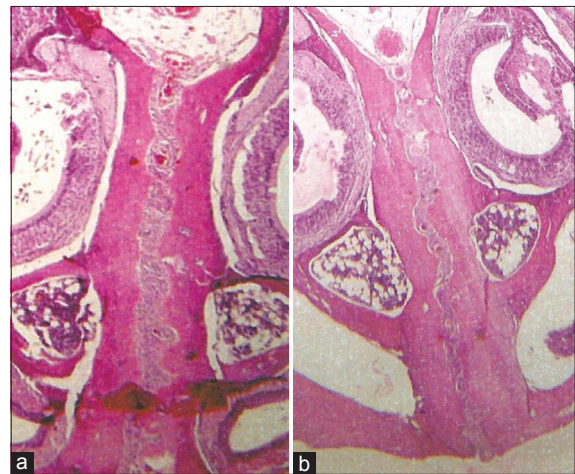
**Diagram 4:** Mean bone regeneration (in 0.001 inch) after 30 days. The difference was significant

inflammation and pain by different mechanisms including increasing levels of some prostaglandins, decreased infection, affecting sensory receptors, and proper coagulation and hemostasis.<sup>[8,11-14,16,17,19,27-38]</sup> A significant difference in weight changes during 1<sup>st</sup> days after placing the appliance in all groups can verify it.

According to findings of this study, a difference of the extent of regenerated bone during the 1<sup>st</sup> week was not significant between laser-included and laser-free groups. This indicates



**Figure 7:** Histological view of midpalatal suture, 14 days after expansion (a) without laser therapy and (b) with laser therapy



**Figure 8:** Histological view of midpalatal suture, 30 days after expansion (a) without laser therapy and (b) with laser therapy during the first 14 days

that the effect of laser does not appear in the 1<sup>st</sup> week. Based on a study of Saito and Shimizu on the same timescale, there is a significant difference in the bone regeneration of groups that received laser therapy compared with control groups.<sup>[4]</sup> Probably, laser power and exposure time can affect the amount of bone regeneration as well.

Difference in bone regeneration in laser-included and laser-free experimental groups was significant in the 14<sup>th</sup> day, which was consistent with another study.<sup>[21]</sup> Pretel *et al.* reported an advanced tissue response and bone formation in the laser group, shortened initial inflammatory reaction and promoted rapid new bone matrix formation in 15 and 45 days. However, they did not find any significant differences between the groups in 60<sup>th</sup> day.<sup>[15]</sup> They concluded that aiming infrared low-level laser directly at the wound showed a biostimulating effect on bone remodeling by stimulating the modulation of the initial inflammatory response and enhancing the recovery.<sup>[15]</sup> Cepera *et al.* as well concluded that low-level laser can affect the bone regeneration process of the suture and accelerate

healing.<sup>[13]</sup> Angeletti *et al.* as well observed a greater and accelerated amount of bone regeneration after rapid palatal expansion.<sup>[39]</sup> The difference in bone regeneration extent in laser-treated and laser-free experimental groups was significant as well. During days of 14–30,  $4 \times 10^{-3}$  inches of regenerated bone is reduced, which was not significant. The reason may be the gradual elimination of inflammatory factors and replacement by osteoblastic phase. Laser exposure can increase amount of ATP, that is, intracellular energy, during primary and secondary cellular reactions. In this process, light energy along primary reactions in oxidation cycle activates the intracellular reactions and enhances the bone healing.<sup>[22,38]</sup>

## CONCLUSION

Low-power laser irradiation can increase and accelerate bone regeneration in the midpalatal suture during and after rapid palatal expansion. While the highest extent of bone regeneration was seen in the first 7 days, the highest efficacy of laser was observed in 3<sup>rd</sup> and 4<sup>th</sup> week. This confirms late effects of laser.

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Nil.

## Conflict of Interest

There are no conflict of interest.

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