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ORIGINAL ARTICLE

An evaluation of the effect of pulsed wave low-level laser therapy on the biomechanical properties of the vertebral body in two experimental osteoporosis rat models

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Abstract Osteoporosis (OP) increases vertebral fragility as a result of the biomechanical effects of diminished bone structure and composition. This study has aimed to assess the effects of pulsed wave low-level laser therapy (PW LLLT) on cancellous bone strength of an ovariectomized (OVX-d) experimental rat model and a glucocorticoid-induced OP (GIOP) experimental rat model. There were four OVX-d groups and four dexamethasone-treated groups. A group of healthy rats was used for baseline evaluations. The OVX-d rats were further subdivided into the following groups: control rats with OP, OVX-d rats that received alendronate, OVX-d rats treated with PW LLLT, and OVX-d rats treated with alendronate and PW LLLT. The remaining rats received dexamethasone and were divided into four groups: control, alendronate-treated rats, laser-treated rats, and laser-treated rats with concomitant administration of alendronate. PW LLLT (890 nm, 80 Hz, 0.972 J/cm^2) was performed on the spinal processes of the T12, L1, L2, and L3 vertebras. We extracted the L1 vertebrae and submitted them to a mechanical compression test. Biomechanical test findings showed positive effects of the PW LLLT and alendronate administration on increasing bending stiffness and maximum force of the osteoporotic bones compared to the healthy group. However, laser treatment of OVA-d rats significantly increased stress high load compared to OVA-d control rats. PW LLLT preserved the cancellous (trabecular) bone of vertebra against the detrimental effects of OV-induced OP on bone strength in rats compared to control OV rats.

Keywords Low-level laser therapy · Glucocorticoid administration · Osteoporosis · Ovariectomy · Alendronate · Biomechanical properties · Rat · Lumbar vertebra · Cancellous bone

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Introduction

Osteoporosis (OP) increases vertebral fragility as a result of the biomechanical effects of diminished bone structure and composition [1, 2]. Wright et al. have reported that the combination of OP and low bone mass at the femoral neck or lumbar vertebrae affected an estimated 53.6 million older US adults in 2010 [3]. Looker et al. analyzed data from the National Health and Nutrition Examination Survey during 2005–2008 and showed that in the USA the prevalence of OP at the femoral neck and lumbar vertebra was 5 and 6 %, respectively [4]. Although the incident rates have stabilized, OP fractures, in particular the hip and vertebra, are associated with considerable expense and increased risk of disability and mortality [5]. Mortality is usually highest during the first year after fracture; however, a notably increased mortality risk might persist for several years after the event. In addition to its efficacy in the prevention of new and recurrent osteoporotic fractures, medical treatment has been associated with improved survival after osteoporotic fractures. In their review article through observational and randomized clinical trials, Sattui and Saag have stated that clinical administration of bisphosphonates not only is effective in the prevention of OP but also increases the survival rates in patients with OP fractures. The rationale behind this administration however remains unclear and necessitates additional research [5].

Various animal models have been used to investigate the pathogenesis of OP as well as facilitate preclinical testing and new treatment options such as anti-resorptive drugs [6]. Histomorphometric parameters and biochemical markers of bone metabolism in animal studies only indicate a decrease in bone formation and minimal changes in bone resorption. These parameters are less important with regards to OP-associated fractures and investigations in orthopedic surgery. Furthermore, histological and biochemical studies do not give direct information about the mechanical strength of the bone. The ultimate reason for fracture of a bone following minimal trauma is the reduction of mechanical strength [7]. Although bone densitometry is frequently used to assess bone fragility, direct biomechanical testing of the bone undoubtedly provides more information about mechanical integrity [8].

Prevention of osteoporotic vertebral fractures can assist millions of at-risk individuals to maintain pain-free independence and long-term health. Current treatments for OP comprise systemic therapies that aim to increase bone mineral density (BMD) and reduce fracture risk [9]. Bone quality encompasses a number of bone tissue properties that govern mechanical resistance such as bone geometry, cortical properties, trabecular microarchitecture, bone tissue mineralization, quality of collagen and bone apatite crystal, and presence of microcracks. These properties all depend on bone turnover and its variations. Decreases in bone resorption markers achieved with resorption inhibitors may partially predict a decrease in fracture risk. At the spine, however, this correlation exists to the level of a 40 % fall in bone resorption markers; in a study, larger declines have not been shown to provide additional protection against fractures in patients who take risedronate. OP medications can exert favorable effects on bone size and cortical thickness. Such effects have been documented with teriparatide (PTH 1–34), which is a unique, purely anabolic treatment for OP. More surprising are the favorable effects on bone size seen with some of the bone resorption inhibitors such as neridronate in adults with osteogenesis imperfecta. Similarly, estrogens and alendronate can increase femoral neck size in postmenopausal women. Preservation of the trabecular microarchitecture has been initially demonstrated with risedronate and subsequently with alendronate [10]. Despite the availability of efficacious treatments for fracture reduction in patients with OP, there are still unmet needs that require a broader range of therapeutics [11].

Several researchers have determined that continuous wave (CW) low-level laser therapy (LLLT) stimulates in vitro mineralization through increased IGF-I and BMP production, Runx2 expression, and ERK phosphorylation [12]. CW LLLT has been shown to stimulate bone nodule formation [13] in osteoblasts. Others reported that LLLT promoted the acceleration of bone strength and consolidation after a fracture, created new blood vessels, increased collagen fiber deposition, and promoted greater bone cell proliferation at the fracture site [14, 15]. Pinheiro et al. reported that LLLT resulted in increased mineralized bone tissue in fractured femora [16]. Bossini et al. concluded that LLLT improved bone repair in the tibia of osteoporotic rats by stimulation of newly formed bone, fibrovascularization, and angiogenesis [17].

Ko et al. evaluated LLLT in treatment of trabecular bone loss induced by skeletal unloading. In that study, mice underwent denervation surgery. After denervation, CW LLLT (wavelength, 660 nm; energy, 3 J) was applied to the tibiae of mice. Ko et al. reported that LLLT might enhance bone quality and bone homeostasis associated with enhancement of bone formation and suppression of bone resorption [18]. In another study, Ko et al. tested the effect of LLLT in prevention and/or treatment of osteoporotic trabecular bone. The tibiae of ovariectomized-induced OP (OVX-d) mice were treated with pulsed wave LLLT (PW LLLT) (660 nm, 3 J). Their results indicated that LLLT might be effective for the prevention and/or treatment of trabecular bone loss. This effect might be site-dependent in the same bone [19]. Diniz et al. studied the influence of CW LLLT in combination with bisphosphonate on OVX-d in cancellous (trabecular) bone of the femoral neck and vertebrae (T13–L2) of rats [20]. Their study divided 35 female rats into five groups: (1) sham-operated rats (control), (2) OVX-d rats with OP, (3) laser-treated OVX-d rats with OP, (4) OVX-d rats with OP treated with alendronate, and (5) OVX-d rats with OP treated with alendronate and laser. Groups 3 and 5 received daily oral alendronate. CW LLLT

(830 nm, 50 mW, and 4 J/cm^2) was administered to the femoral neck and spinal vertebra in groups 4 and 5. Rats from the OP control and OP + laser groups showed marked OP. In the OP + bisphosphonate group, there was significantly more cancellous bone volume in the vertebra than in the OP control group. Notably, in the association between laser and alendronate, the cancellous bone volume was significantly greater in the vertebrae. This finding was similar to the sham-operated control group. Diniz et al. concluded that laser therapy associated with alendronate treatment was the best method for reversing vertebral OP caused by an ovariectomy [20].

Evidence exists that pulsed light dose has effects that differ from CW [21]. The use of pulsed light is increasing and a review of literature has shown three cellular studies on rat calvarial cells [22–24], one in vivo study on the tooth movement speed of rat molars [25], one study on bone turnover in OVX-d rats [26], and one study on healing of partial tibial osteotomy in streptozotocin-induced diabetic rats [27].

The aim of this study was to assess the effects of PW LLLT on cancellous bone strength of an OVX-d experimental rat model and a glucocorticoid-induced OP (GIOP) experimental rat model. We evaluated bone strength by measuring the biomechanical properties of the first lumbar vertebral body which included bending stiffness (Young modulus of elasticity), maximum force, and stress high load.

Materials and methods

Experimental animals

A total of 54 adult male and female Wistar rats, aged 4.5 months, were housed in standard rat cages in a 12-h light/dark environment. Rats received water ad libitum. All experimental procedures were approved by the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (protocol no 1391-1-115-1092). The rats' body weights were monitored weekly, and the volume of drugs administered was calculated according to the most recent body weights.

Study design

OVX-d rats and GIOP rats received PW LLLT and alendronate, after which they were subjected to a mechanical compression test.

Ovariectomized-induced osteoporosis (OVX-d) and GIOP rats

We randomly assigned the 54 rats into nine groups of six rats each as follows: four OVX-d groups, four groups that received dexamethasone, and one healthy group that was considered for baseline studies (H, group 5). Ovariectomies were performed via two paravertebral skin incisions made when the rats were anesthetized with ketamine (50 mg/kg, i.m.) and diazepam (5 mg/kg, i.m.) [28, 29]. The uterine tubes were ligated (catgut 4.0) and, following removal of the ovaries, the incisions were closed (nylon 3.0). Antibiotic therapy with ceftriaxone (Jaber ben Hayan, Tehran, Iran) at a dose of 50 mg/kg was administrated immediately before surgery and at 24 and 48 h after surgery. All animals were kept for 14 weeks after surgery in cages in order to develop OP [28, 29]. At the end of this period, rats were submitted to the following treatments: group 1, control rats with OP (OC); group 2, OVX-d rats treated subcutaneously [28] with 1 mg/kg alendronate [30] (Alborz Darou, Tehran, Iran) for 30 days (OA); group 3, OVX-d rats treated with PW LLLT (OL); and group 4, OVX-d rats treated with PW LLLT and concomitant administration of alendronate (OAL).

In the current study, the surface area of the target tissue was larger than the pen's spot size; therefore, we used sequential treatments to ensure that each unit area received a similar laser dose [31]. PW LLLT was performed on the spinal processes of T12, L1, L2, and L3 vertebrae with the laser pen held perpendicular to the target tissue at a distance of <1 cm. During PW LLLT, animals were sedated by administration of 1/2 the dosage of the anesthesia drugs. PW LLLT was administered for 3 days per week for an 8-week period. The specifications of the laser used are shown in Table 1 [29, 32].

The remaining rats received intramuscular injections of dexamethasone (Alborz Darou, Tehran, Iran) at 1 mg/kg/day administered 6 days/week for a 5-week period as a modified protocol [33]; the referred drug administration was modified to 6 days per week instead of 7 days per week and 5 weeks instead of 4 weeks. After 5 weeks, we divided the rats into four groups: group 6, control OP rats treated with intramuscular injections of vehicle (distilled water: DC); group 7, GIOP rats treated with subcutaneous injections of 1 mg/kg alendronate (Alborz Darou, Tehran, Iran; DA); group 8, GIOP

Table 1 Specifications of the infrared laser used

Parameters	Dose and unit
Peak power output	75 W
Average power	1.08 mW
Power density	1.08 mW/cm^2
Wavelength	890 nm
Pulse frequency	80 Hz
Spot size	1 cm^2
Pulsed duration	180 μs
Duration of exposure for each point	900 s
Energy density	0.972 J/cm^2

rats treated with PW LLLT (DL); and group 9, GIOP rats treated with PW LLLT and concomitant administration of alendronate (DAL).

General examinations

At 8 weeks after the beginning of laser treatment and alendronate administration, the rats were killed with an overdose of anesthesia. Blood glucose concentrations were measured using distal tail vein blood samples (GM 300, Biomince, GMH, Heerbrugg, Switzerland). We extracted the laser-treated vertebrae (T12–L3). The height of the first lumbar vertebra (mm) was measured using a sliding caliper and its weight (g) measured. T12, L2, and L3 vertebrae were frozen for future analyses.

Biomechanical examinations

Biomechanical properties of the first lumbar vertebral body bone were determined by the uniaxial compression test which was performed using a material testing machine (Z 2.5, Zwick Gm Bh & Co., Ulm-Einsingen, Germany). The rate of the compression test was 5 mm/min, and we obtained the load–deformation curve. In order to evaluate biomechanical behavior, we measured bending stiffness (Young modulus of elasticity; N/mm), maximum force (N), and stress high load (N/mm^2).

Bending stiffness is the slope of the linear portion of the load–deformation curve (the ratio of loading to deformation in the elastic region of the curve). Maximum force or ultimate compression strength is the maximum load that is borne by the first vertebral body bone. The stress high load was calculated by dividing the maximum force by the bone's surface area (mm^2).

Statistical analysis

All data were expressed as mean ± standard errors of mean (SEM).

Normal distribution of data was analyzed by the one-sample Kolmogorov-Smirnov test. Parametric and nonparametric statistical methods were used. The analysis of variance (ANOVA) test was used to compare changes among groups with normal distribution of data and the least significant difference (LSD) test to identify differences. A p value of ≤ 0.05 was considered statically significant. Nonparametric methods were used for statistical analysis of other groups. These data were analyzed using the Kruskal-Wallis and Mann-Whitney U tests. Differences were regarded as significant if $p < 0.005$ for analyses between groups 1 and 9. The differences were also regarded as significant if $p \leq 0.01$ for analyses between groups 1 and 5 and between groups 5 and 9.

Results

General observations

Blood glucose levels

According to the ANOVA test, there were no significant differences in blood glucose levels between the studied groups (Table 2).

Body weight

The Student's t test showed that administration of dexamethasone led to a significant decrease in body weight ($p < 0.001$). Although we observed weight loss in all groups that received dexamethasone, the weight loss was more severe in rats from groups DC and DL.

In all these groups, five rats died during dexamethasone administration and three died during alendronate treatment and PW LLLT. In the OVX-d groups, four rats died during the study. The dead rats were replaced.

However, treatment with alendronate in rats that had received dexamethasone completely prevented dexamethasone-induced weight loss. Laser treatment of dexamethasone-treated rats also prevented most of the dexamethasone-induced weight loss ($p < 0.05$). Alendronate treatment of OV rats significantly increased body weight (Student's t test, $p < 0.05$; Table 3).

Height of L1 vertebra

According to the LSD test, the L1 vertebra height of DC, DA, OAL, DAL, OC, OL, and OA groups significantly compared to the healthy group ($p = 0.000$, $p = 0.000$, $p = 0.009$, $p = 0.016$, $p = 0.027$, $p = 0.032$, and $p = 0.043$, respectively). PW LLLT with concomitant administration of alendronate increased the height of the L1 vertebra among the studied groups. There was significant difference between DAL and DL groups ($p = 0.008$). Alendronate treatment of OVX-d rats decreased the L1 vertebra height among the studied groups. There was significant difference between OA and DA groups ($p = 0.001$). Significant differences are shown in Fig. 1.

Weight of L1 vertebra

The LSD test showed that the L1 vertebra weight from DC, DA, DAL, OC, OAL, OL, DL, and OA groups significantly increased compared to the healthy group ($p = 0.000$ for first six groups and $p = 0.001$ and $p = 0.012$ for DL and OA groups, respectively). Administration of alendronate to dexamethasone-treated rats increased the weight of the L1 vertebra compared to DL, OL, OA, DAL, DC, OC, and OAL groups ($p = 0.000$, $p = 0.002$, $p = 0.002$, $p = 0.003$,

Table 2 Mean ± SEM of blood glucose (ml/dc) of the groups compared by ANOVA test

Groups	OC	AO	OL	OAL	DC	DA	DL	DAL
Blood glucose	113 ± 3.06	114.5 ± 2.48	114.5 ± 3.03	112.2 ± 8	114.7 ± 2.56	115.3 ± 2.14	114.4 ± 4	113.5 ± 2.7

OC ovariectomized (OVX-d) control rats, *OA* OVX-d rats treated with alendronate, *OL* OVX-d rats treated with LLLT, *OAL* OVX-d rats treated with LLLT and alendronate, *H* healthy rats, *DC* dexamethasone-treated control rats, *DA* dexamethasone-treated rats that received alendronate, *DL* dexamethasone-treated rats that received PW LLLT, *DAL* and dexamethasone-treated rats that received alendronate and PW LLLT

$p = 0.02$, $p = 0.02$, and $p = 0.036$, respectively). Laser treatment of dexamethasone-treated rats decreased the weight of the L1 vertebra among the studied groups. Significant differences are shown in Fig. 2.

Biomechanical results

PW LLLT and alendronate increased bending stiffness and maximum force of the osteoporotic bones compared with the healthy group. Laser treatment of OVA-d rats also significantly increased the high stress load compared to OVA-d control rats.

Bending stiffness

Alendronate administration of OVA-d rats showed the highest value for bending stiffness among the studied groups. Laser treatment of dexamethasone-treated rats showed a significant difference in bending stiffness of the L1 lumbar vertebra compared to the healthy group (Mann-Whitney test; $p = 0.004$; Fig. 3).

Maximum force

Laser treatment of the L1 lumbar vertebra from OVA-d rats increased the maximum force compared to the other groups. There were significant differences in terms of maximum forces in the OL ($p = 0.01$), OA ($p = 0.025$), and DL ($p = 0.029$) groups compared to the healthy control group (LSD test; Fig. 4).

High stress load

According to the Mann-Whitney test, a comparison of the high stress load of the L1 lumbar vertebra between the OVX-d control group and healthy group was approximately significant ($p = 0.037$). Laser treatment of the L1 lumbar vertebra from OVA-d rats increased the high stress load compared to the OVA-d control group (Mann-Whitney test, $p = 0.01$). Alendronate treatment of dexamethasone-treated rats increased the high stress load of the L1 lumbar vertebra compared to the healthy group (Mann-Whitney test; $p = 0.006$; Fig. 5).

Discussion

The current study results showed significant weight loss and approximately 10 % mortality rate among the dexamethasone-treated rats. Some biomechanical properties of the L1 vertebrae from the dexamethasone-treated rats were comparable with those of the healthy rats and PW LLLT-treated rats. PW LLLT acted as an anabolic agent on bones [34].

These findings might be attributed to the anabolic effects of GC administration at the level of the cancellous bone. These data were markedly distinct from the findings in patients with supraphysiologic GC administration who had bone loss [35]. Supraphysiologic doses of GC have additionally caused an increase in patients' body weights [36]. The anabolic effects of GC administration on the cancellous bone as reported in the current study were consistent with previous studies [37, 38]. In contrast to the effects

Table 3 Mean ± SEM of initial body weight and last body weight of all groups

Groups → Weights ↓	OC	OA	OL	OAL	DC	DA	DL	DAL
INITIAL	231.2 ± 4.3	236.4 ± 4.5	240 ± 4.1	237.6 ± 4.7	244.7 ± 6.9	232 ± 4.2	246 ± 4.2	234.7 ± 5.9
LAST	232 ± 3.5	247.4 ± 3.3**	233 ± 4.6	232.3 ± 3.6	220.8 ± 5.8***	228.7 ± 6.7	238.8 ± 2.8*	230 ± 3.9

Student's *t* test showed significant differences between initial and last body weight of OC, DC, and DL groups

OC ovariectomized (OVX-d) control rats, *OA* OVX-d rats treated with alendronate, *OL* OVX-d rats treated with LLLT, *OAL* OVX-d rats treated with LLLT and alendronate, *H* healthy rats, *DC* dexamethasone-treated control rats, *DA* dexamethasone- treated rats received alendronate, *DL* dexamethasone-treated rats received LLLT, *DAL* dexamethasone-treated received alendronate and LLLT

**$p < 0.01$, *$p < 0.05$, *$p < 0.001$

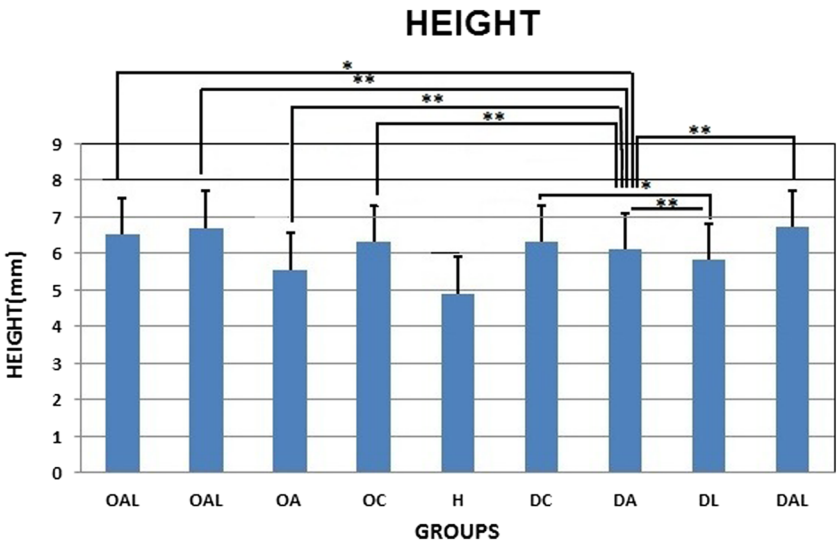

Fig. 1 Mean ± SEM of L1 vertebra height (mm) of the studied groups compared by the LSD test; $p < 0.05$, $p < 0.01$, $p < 0.001$. According to the LSD test, the L1 vertebra height of DC, DA, OAL, DAL, OC, OL, and OA groups significantly compared to the healthy group ($p = 0.000$, $p = 0.000$, $p = 0.009$, $p = 0.016$, $p = 0.027$, $p = 0.032$, and $p = 0.043$, respectively). PW LLLT with concomitant administration of alendronate increased the height of the L1 vertebra among the studied groups. Alendronate treatment of OVX-d rats decreased the L1 vertebra height among the studied groups

of GC administration on the biomechanical properties of vertebral body in the current study, several studies reported that biomechanical properties of the vertebral bone and cancellous bone tissue decreased following GC administration [39, 40]. The cellular mechanisms underlying the anabolic effects of GC administration on the cancellous bone of the vertebral bone that has been observed in the current study are not clear.

Renno et al. investigated the effects of CW LLLT (830 nm) on the femurs of exercised OP rats. The exercised animals showed higher bone strength and physical property values. However, CW LLLT did not improve the stimulatory effects of the exercise on OP rats [41]. Diniz et al. reported that CW laser therapy associated with alendronate treatment was the best method for reversing vertebral OP caused by an ovariectomy [20].

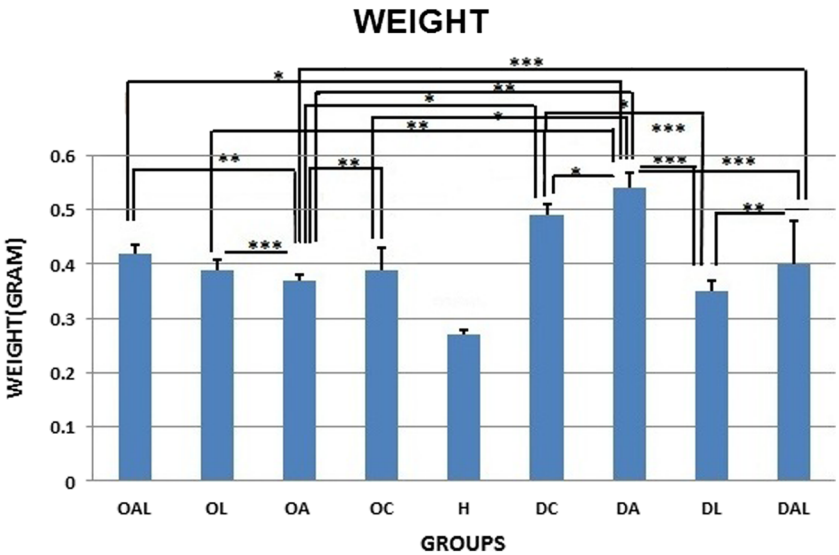

Fig. 2 Mean ± SEM of L1 vertebra weight (g) of the studied groups compared by the LSD test; $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. The LSD test showed that the L1 vertebra weight from DC, DA, DAL, OC, OAL, OL, DL, and OA groups significantly increased compared to the healthy group ($p = 0.000$ for first six groups and $p = 0.001$ and $p = 0.012$ for DL and OA groups, respectively). Administration of alendronate to dexamethasone-treated rats increased the weight of the L1 vertebra compared to DL, OL, OA, DAL, DC, OC, and OAL groups ($p = 0.000$, $p = 0.002$, $p = 0.002$, $p = 0.003$, $p = 0.02$, $p = 0.02$, and $p = 0.036$, respectively). Laser treatment of dexamethasone-treated rats decreased the weight of the L1 vertebra among the studied groups

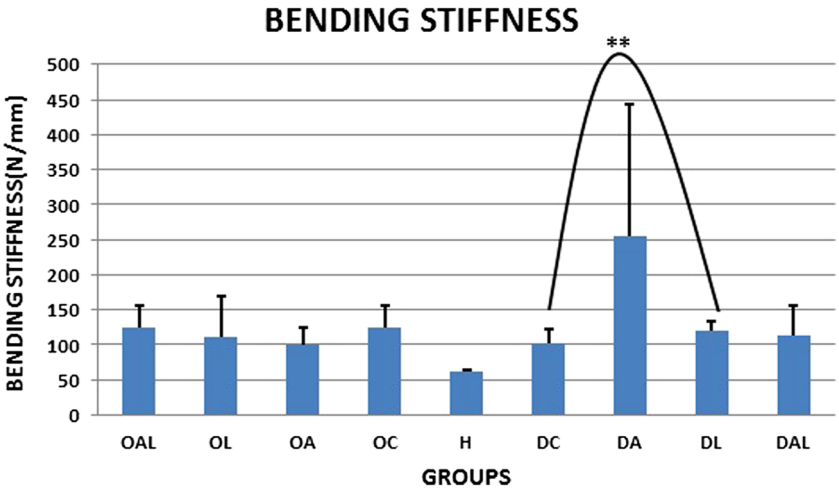

Fig. 3 Mean ± SEM of L1 vertebra bending stiffness (N/mm) of the studied groups compared by the Mann-Whitney test. $^{**}p < 0.01$. Alendronate administration of OVA-d rats showed the highest value for bending stiffness among the studied groups. Laser treatment of dexamethasone-treated rats showed a significant difference in bending stiffness of the L1 lumbar vertebra compared to the healthy group

The biomechanical results of the current study showed the positive effects of PW LLLT and alendronate administration on increasing maximum force of both models of osteoporotic bones compared to the healthy group. In the OVX-d rats, the biochemical analysis results showed the stimulatory effects of PW LLLT on stress high load of OVX-d rats compared to OVX-d control rats. Of note, in the current study, PW LLLT was more effective than alendronate in preserving bone from the detrimental effects of an ovariectomy on cancellous bone. Consistently, Renno et al. investigated the effects of infrared CW LLLT alone on the femora of OVX-d rats [42]. They divided 60 female rats into six groups: sham-operated control (SC), osteopenic control (OC), sham-operated irradiated at a dose of 120 J/cm^2 (I120), osteopenic irradiated at a dose of 120 J/cm^2 (O120), sham-operated irradiated at a dose of 60 J/cm^2 (I60), and osteopenic irradiated at a dose of 60 J/cm^2 (O60). Laser irradiation, initiated 8 weeks after surgery, was performed three times per week for 2 months. The femurs were submitted to a biomechanical test. The results indicated that the maximum load of O120 did not show any difference when compared with the SC and I120 groups; however, it was higher than the O60 group. Renno et al. concluded that photoradiation had stimulatory effects on the femora of osteopenic rats. This effect was mainly observed at a dose of 120 J/cm^2. However, future studies should investigate the effects of different parameters, wavelengths, and sessions of LLLT on OVX-d rats.

The mechanism of action of PW LLLT in bones is an important area of research. Thus far, the precise roles of PW LLLT in bone remodeling are still not fully understood [21]. In recent years, a number of studies have examined the specific effects and mechanism of action of PW LLLT by using in vivo and in vitro models [22–27]. In vitro studies have shown positive effects of 1 Hz pulse frequency and 830 nm on bone nodule formation [22]; 0.48–3.84 J/cm^2, 1–8 Hz, and 830 nm on bone nodule formation in rat calvarial cells [24]; and 1.1 and 2.2 J/cm^2, 6000 Hz, and 650 nm on RANKL and OPG mRNA expression in rat calvarial cells [23]. Ueda et al. reported that laser irradiation with 2 Hz was the most effective parameter for stimulating bone nodule formation [24]. Other

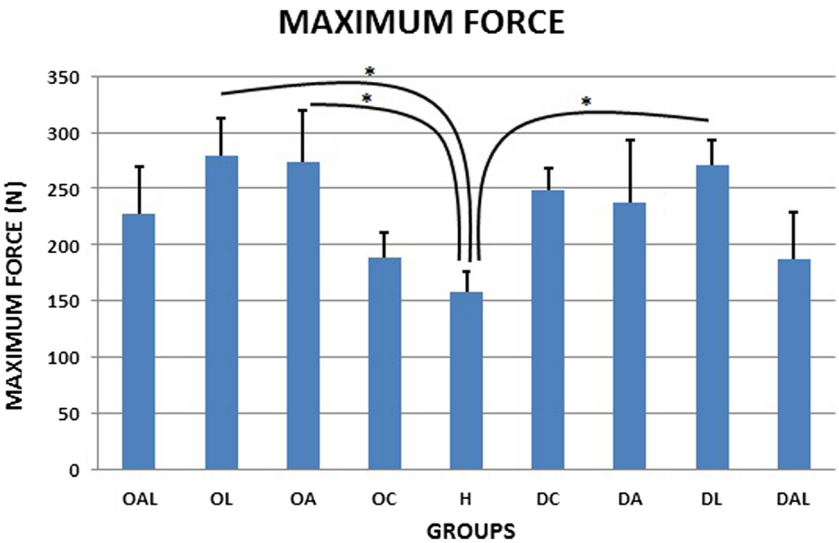

Fig. 4 Mean ± SEM of L1 vertebra maximum force (N) of the studied groups compared by LSD test; $p < 0.05$. Laser treatment of L1 lumbar vertebra of the OVA-d rats increased maximum force compared to the other groups. Maximum force of the OL, OA, and DL groups showed significant differences compared to the healthy group

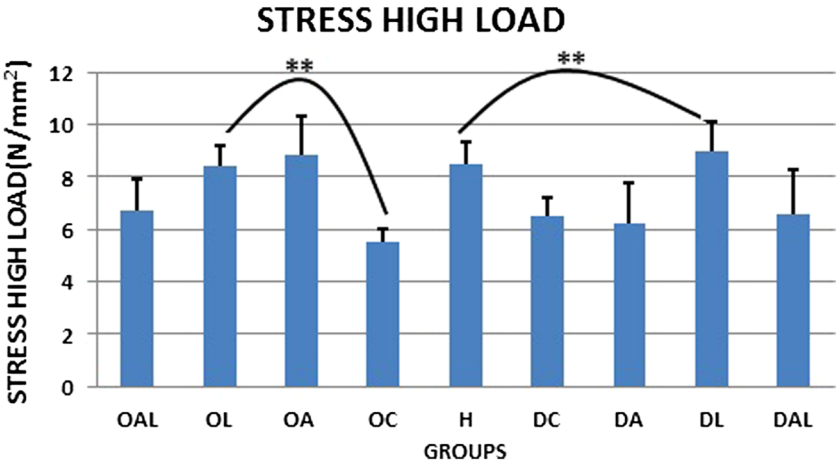

Fig. 5 Mean ± SEM of L1 vertebra high stress load (N/mm^2) of the studied groups compared by the Mann-Whitney test; $^{**}p < 0.01$. Statistical analysis showed that the difference in stress high load of L1 lumbar vertebra between the OVX-d control group and healthy group approximated a significant difference ($p = 0.037$). Laser treatment of the L1 lumbar vertebra of OVA-d rats increased high stress load compared to the OVA-d control group. Alendronate treatment of dexamethasone-treated rats increased the high stress load of L1 lumbar vertebra compared to the healthy group

in vivo studies reported the stimulatory effects of 3.6 J/cm^2, 2–8 Hz, and 830 nm on the tooth movement speed of rat molars [25] and 11.6 J/cm^2, 3000 Hz, and 890 nm on healing of a partial tibial osteotomy in streptozotocin-induced diabetic rats [26].

The bio-stimulatory effect of PW LLLT has many potential clinical applications and is a significant research topic in laser medicine. OP is the most common bone disease which leads to a consequent increase in fractures among the elderly. It is well known that osteoporotic fractures decrease quality of life and increase mortality rates in the older population [43]. However, at present, there are few effective therapies and medications available for long-term treatment and prevention of this chronic disease [44]. PW LLLT irradiation can promote bone formation and inhibit bone resorption, thus facilitating bone remodeling. This technique may be a potential therapy for the treatment of OP in patients.

The physical parameters used in PW LLLT such as wavelength, power (peak power, average power, and power density), pulse frequency, pulse duration (width), duration of exposure, and energy density all impact the biological effects of laser irradiation [23, 45].

The current study examined the effects of PW LLLT irradiation of a relatively low energy density compared to other in vivo studies [25, 27], a high peak power, and long duration for each shooting at the cancellous bone tissue level.

Conclusion

PW LLLT at an energy density of 0.972 J/cm^2 and frequency of 80 Hz preserved the cancellous bone of vertebra against detrimental effects of ovariectomy-induced OP on bone strength in rats compared with control OV rats. Our results showed that PW LLLT of OVX-d rats led to better biomechanical results when compared with OV rats that received alendronate. The cellular, biochemical, and molecular mechanisms regarding the effects of PW LLLT on osteoporotic cancellous bone should be elucidated by conducting additional studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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