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ASSESSMENT OF THE HEALING EFFECT OF SIMVASTATIN ON INDUCED PERIAPICAL LESIONS IN DOGS

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ABSTRACT

The most prevalent disease in the jaw is the chronic inflammatory periapical lesion. The lack of impact of calcium hydroxide dressing on healing results suggests that more effective inter-appointment dressings should be developed. Simvastatin is one of the most widely used pharmaceuticals in the world today as means of combating high cholesterol. Simvastatin's osteopromotive capabilities is being studied, however its effect on healing of periapical lesions remains unclear. **Materials and methods:** Periapical lesions were induced in 64premolars in four Mongrel dogs. Dogs of one **group** were injected through the root canals with **2.5mg/ml simvastatin**, while dogs of another **group** were injected with **5mg/ml simvastatin**. Dogs of the control group were not injected. The animals were sacrificed after 2 and 4weeks. Bone formation was evaluated by H&E stain, Masson-Goldner trichrome stain and osteopontin immunostain. Area percent for Masson-Goldner trichrome and osteopontin stain was calculated. **Results:** The area percent of Masson-Goldner trichrome and osteopontin expression were enhanced by the dose and time of exposure to the drug. **Conclusion:** Although, the most important part of treating apical periodontitis is a full root canal therapy, however simvastatin improved the efficacy of this procedure.

KEY WORDS: Simvastatin, periapical lesion, osteopontin, bone healing

INTRODUCTION

Most occurrences of apical periodontitis are caused by an infection that spreads down the intraradicular canal. Endodontic therapy is crucial because it removes the infectious pathogens and let the patient to recover. When it comes to the jaw's alveolar bone, the most prevalent disease is the chronic inflammatory periapical lesion [1].

Endodontic treatment has been shown to reduce inflammatory mediators, proinflammatory cytokines, and growth factors, which may halt the proliferation of epithelial cells in epithelial strands

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of periapical granulomas and the lining epithelium of apical cysts. Apoptosis, or programmed cell death, may be triggered in epithelial cells when they are deprived of survival factors or when they are exposed to death signals during periapical wound healing [2].

The lack of impact of calcium hydroxide dressing on disinfection of the root canal system and treatment result suggests that more effective inter-appointment dressings should be developed. [3].However, some studies found that periapical healing is enhanced by using a calcium hydroxide paste dressing rather than a single-session root canal filling [4,5,6].

Ozonized oil might also potentially be used as an intracanal medication[7]. When compared to traditional nonsurgical endodontic therapy, the novel Apexum method was shown to have the potential to greatly increase the healing kinetics of apical periodontitis [8].

Large periradicular lesion in a tooth may be treated endodontically without surgery. After conventional treatments failed to alleviate symptoms, researchers found that a paste containing metronidazole, ciprofloxacin, and minocycline was effective in treating teeth with extensive periradicular lesions[9].

Since the 1990s, simvastatin has been studied extensively together with other pharmaceutical substances for its osteopromotive effects. Simvastatin is a member of the statin family of medications, which are chemically similar to 3-hydroxy-3-methylglutaryl-coenzyme A. (HMG-CoA). Statins may be able to reversibly inhibit HMG-CoA reductase by blocking the enzyme's substrate-product transition state through side chains that bind to the active site. They were first created as a means of combating high cholesterol. Pravastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin, pitavastatin, and rosuvastatin are all examples of -statin medications that may be purchased over the counter. One of the most often given medications is simvastatin [10].

Mevalonate is the precursor to both cholesterol and isoprenoid compounds; these lipid attachments are necessary for the correct localization and activation of many different proteins, including monomeric Guanine triphosphatases (GTPases) like Rho, Rac, and Ras. Statins function primarily by inhibiting the formation of mevalonate. Activation of reactive oxygen species and nuclear factor kappa B (NF-kB) and inhibition of endothelial nitric oxide synthase are only two examples of the inflammatory responses that are mediated by the GTPases[11,12,13].

Accordingly, statins can reduce IL-6 synthesis and NF-kB activation in response to lipopolysaccharides (LPSs). In addition, statins reduce the production of inflammatory cytokines in monocytes[11,14]. There are already a variety of statins on the market, each with their own unique pharmacokinetic features [15]. Because of its widespread usage, simvastatin was chosen for this research.

Studies by Mundy et al. in 1999 were a major step forward in demonstrating the osteoblast-stimulating effects of simvastatin. In a mouse model with a calvaria deficiency, they found that simvastatin promoted bone production and accelerated bone regeneration [16]. It has been hypothesised that the osteoblast-promoting, anti-inflammatory, osteoclast-inhibiting, and neovascularization activities of simvastatin are responsible for simvastatin-mediated bone repair [17, 18]. Inducing bone morphogenic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) gene expression to accelerate the development of osteoblastic cells is one mechanism by which simvastatin exerts its pleiotropic effects on bone metabolism [19]. Moreover, simvastatin was found to retard bone resorption by suppressing cathepsin K expression, block the fusion of osteoclast precursors, and the number of active osteoclasts was decreased[20].

In the past, researchers paid little attention to statins' direct impact on inflammation-induced bone loss. Statins have been studied for their effects

on periodontitis [21, 22], the effects of statins on the progression of periapical lesions remain unclear [23].

MATERIALS AND METHODS

Selection of animals and preoperative care:

Sixty-four premolars in four Mongrel dogs, with an average weight of 20 kgs and an average age of one year, were used for this study.

Preparation of the animals

The animals were starved for (12-24 hours) and kept thirsty for six hours prior to anesthesia. The skin of the maxillary and mandibular regions was clipped and shaved carefully, then washed with water and soap and painted with Betadine.

Premedication

Dosage of 0.05 mg/kg body weight of atropine sulphate was given subcutaneously (10-30 minutes) before surgical procedures.

Induction of anesthesia

Anesthesia was induced using a combination of XylazineHCLc 1 mg/kg body weight and Ketamine HCL5 mg/kg body weight. A 23-gauge IV cannula was inserted into the patient's cephalic vein, and the mixture was injected.

Maintenance of general anesthesia:

An intravenous drip of 1/2 g of thiopental in 500 millilitres of dextrose, 5%, was administered at a rate of 28-40 drops per minute to keep the patient under anaesthesia for the duration of the surgery. The endotracheal tube was used to maintain a clear airway for breathing.

Experimental procedure:

1. Induction of periapical lesion:

Supine on the operation table, the animal had its lips opened with a special mouth gag and its head tilted to clear its airway. Towels that had been disinfected were used to cover the animal. A spherical bur of size 3 was used to create the access cavity, and a fine tapering stone was used for the flare. Starting with a no. 20 K-file, the canals were thoroughly examined before being prepared for pulp removal using a no. 30 or 40 Hedströem file, depending on canal diameter. Radiographic measurements helped establish the usable length. In order to cause periapical lesions in the test subjects, they were left exposed to the oral environment for 30 days. On radiograph, periapical lesions were shown to be progressing.

2. Mechanical preparation and injection of simvastatin

K-files were used to expand the root canal until smooth dentinal shavings were created. A sterile saline solution was used to flush the canals during this treatment. Gates The first two-thirds of the root canal was cleaned and shaped using Glidden drills of sizes 2 and 3, while the latter third was cleaned and shaped with K-files.

Dogs of one **experimental group** were injected through the root canals with **2.5mg/ml simvastatin**, while dogs of another **experimental group** were injected through the root canals with **5mg/ml simvastatin**. Dogs of the control group were not injected.

Intermediate Restorative Material (IRM), a thick rapid setting cement based on zinc oxide and eugenol cement, was used to seal all of the teeth used in the experiments. Use of this substance prevented the passage of microorganisms.

3. Obturation of the root canals:

One week after mechanical preparation and injection of simvastatin, the master cone was selected according to the master apical file which was either no. 60, 70 or 80 according to the age of the dog (young dogs age <1 year had the master apical file no. 80 K-file).

A zinc-oxide and eugenol root canal sealer was properly mixed. Before insertion of the sealer, the canals were dried with paper points. The gutta-percha was then rolled in the mixed cement and gently placed into position with tweezer. The auxillary cones were then inserted into the canals using the lateral compaction technique. Excess gutta-percha was removed with a hot condenser. The access cavities were then sealed with IRM. Radiographs were taken during the procedure to verify the periapical lesions and root canal length.

After surgery, the animals spent a week on a soft diet before switching to normal food.

Radiologic examination:

Immediate postoperative radiographs were performed to check up the quality of the root canal filling.

4. Animal sacrifice:

The animal groups were sacrificed after 2 and 4 weeks by injecting an overdose of Thiopental sodium via a cannulated cephalic vein. After the animalswere sacrificed, skin and subcutaneous tissue were stripped off; the mandible and maxilla of the right side were disarticulated, split and the specimens were fixed in a 10% formalin solution. Scheduling frequent decalcification and staining of collected samples was a standard practice.

Preparation for microscopic examination:

Decalcification:

Block resection containing the treated teeth from the dog's mandible were placed in formalin 10% for one week. Henceforward the specimens were transferred to the decalcifying solutiong (RDO-Apex Engineering Products Corporation, IL-USA). After two weeks the specimens were ready to be processed in a series of graded alcohols and xylene then waxed in paraffin.

Tissue staining

Bone formation was evaluated by H&E stain[1], Masson-Goldner trichrome histochemical stain[7] and osteopontin immunostain[10]. Sections will be examined under the ordinary light microscope.

Histomorphometric evaluation(Image Analyzer)

In order to assess all of the stained sections, the computer system will use the image analyzer software Leica Qwin 500 (Germany) Leica Microsystems LTD. CH9435 Meerbrugg. Serial number 0557060916, Country of Origin: Switzerland, Input Voltage: 12V, Current: 170mA, Model Number: DFC295 (12730469) Three fields from each slide displaying the best typical tissue quality in each group were assessed at a magnification of 400 at a standard measuring frame of 192000 pixels for both Masson-Goldner trichrome stain and osteopontin immunohistochemical staining (x100). For statistical purposes, we shall calculate the average percentage of covered area for each group.

Statistical analysis

The collected information was shown as group means and standard deviations. The unpaired student t-test was used to determine the significance of the differences between the groups. If the probability level was less than 0.05, it was regarded significant, and if it was less than 0.001, it was called very significant. IBM's Statistical Package for the Social Sciences (SPSS) was used for the statistical study[10].

RESULTS

Microscopic examination

H and E stained sections

Microscopic examination of H&E stained sections of the control group after 2 weeks revealed necrosis and abscess formation in the periapical area (fig.1a). The 2.5mg group after 2 weeks showed partial closure of the apical foramen with hard dental tissues.The apical periodontal ligament

space showed extensive widening as marked by the black lines spanning the PL width. Micro abscess formation and widespread foci of edema were also noted (fig. 1b). The 5mg group after 2 weeks revealed complete apical closure, increased periodontal ligament thickness and new periapical bone formation with irregular sized and shaped marrow cavities (fig. 1c).

The control group after 4 weeks demonstrated a wide apical foramen with minor mineralized hard tissues. Necrosis is accentuated together with vasodilated blood vessels (fig. 1d). The 2.5mg group after 4 weeks demonstrated a partially closed apical foramen with minor mineralized hard tissues. Necrosisis emphasized as well as vasodilated blood vessels and intense inflammatory cell infiltrate in the periapical area (fig.1e). The 5mg group after 4 weeks showed complete apical closure as well as enhanced bone formation. The healing activity was perceived except for some mild inflammatory reaction which can still be distinguished in the tissues (fig. 1f).

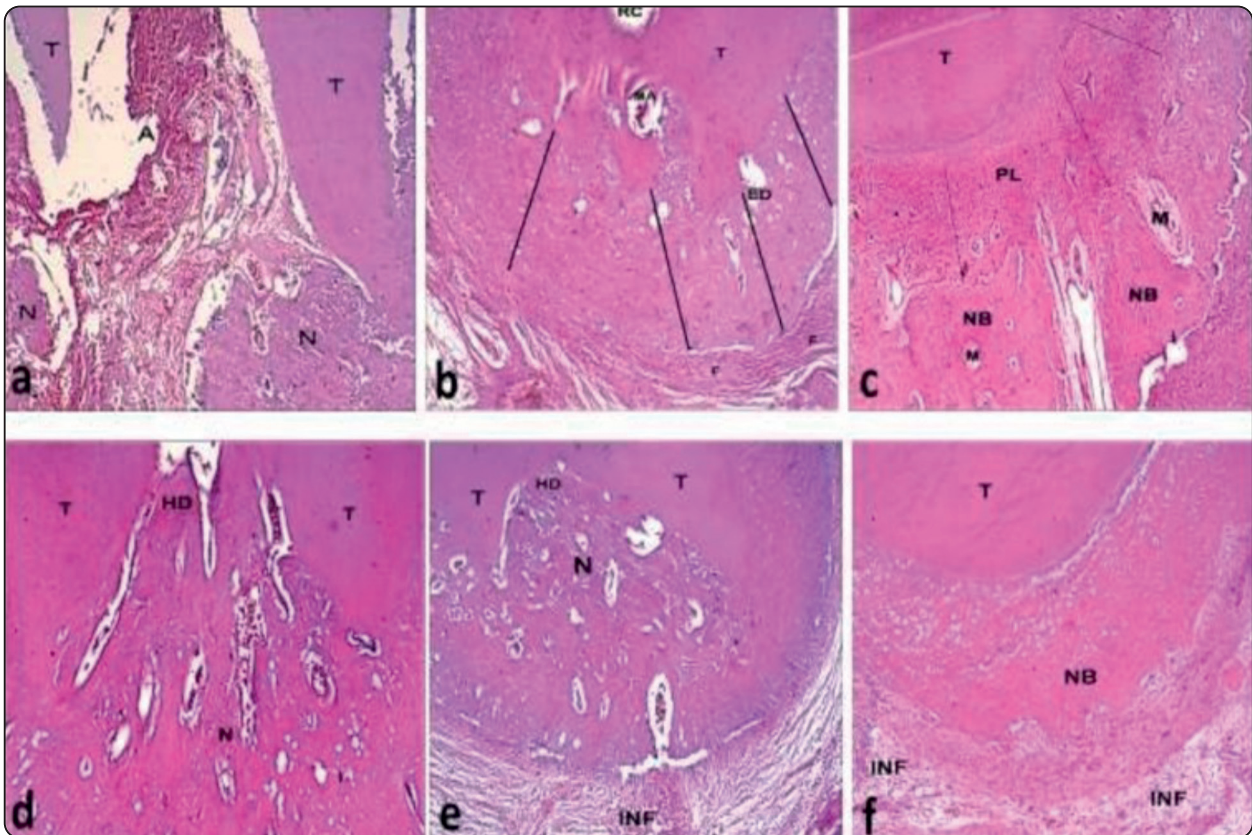

Fig. (1): Photomicrograph of (a) the control group after 2 weeks revealing extensive periapical abscess (A) formation extending into the apical foramen. Note the widespread necrotic tissue,T : TOOTH, (b) the 2.5mg group after 2 weeks showing partial closure of the apical foramen with hard dental tissues (T). The apical periodontal ligament space showed extensive. Note the microabscess formation (MA) and the widespread foci of edema (ED), RC: Root Canal, (c) the 5mg group after 2 weeks revealing complete apical closure (T), increased periodontal ligament thickness (PL) and new periapical bone formation (NB). Note the irregular sized and shaped marrow cavities (M), (d) the control group after 4 weeks demonstrating the wide apical foramen with minor mineralized hard tissues (HD). Necrosis is accentuated together with vasodilated blood vessels spanning the periapical foramen, (e) the 2.5mg group after 4 weeks demonstrating partially closed apical foramen with minor mineralized hard tissues (HD). Necrosis (N) is emphasized as well as the vasodilated blood vessels. Note the intense inflammatory cell infiltrate in the periapical area (INF), (f) the 5mg group after 4 weeks showing complete apical closure as well as an enhanced bone formation (NB) and mild inflammation in the tissues (INF). (H&EX40).

Masson-Goldner Trichrome stained sections

Microscopic examination of Masson-Goldnertrichrome stained section of the control group after 2 weeks showed severe destruction in the form of abscess formation and spicules of bone without any osteocytic lacunae (fig.2a). The 2.5 gm group after 2 weeks showed minute hard tissue formation in the vicinity of the tooth (fig. 2b). The 5 mg group after 2 weeks showed relative increase in new bone formation (fig. 2c).

The control group after 4 weeks showed heavy necrotic tissues in between the widely open apical foramen (fig. 2d). The 2.5 mg group after 4 weeks revealed newly formed bone with wide osteocytic lacunae and osteoblastic rimming (fig. 2e). The 5mg group after 4 weeks showednewly formed periapical bone (fig. 2f).

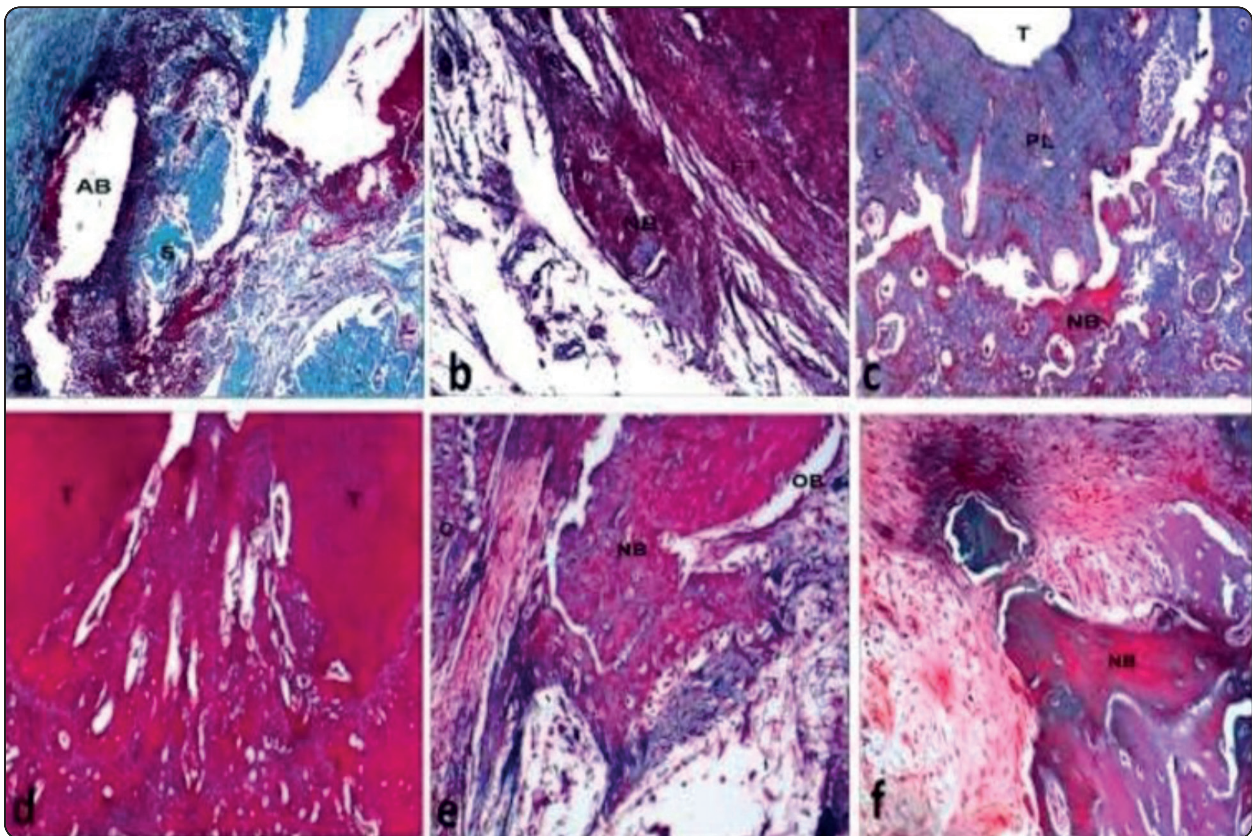

Fig. (2): Photomicrograph of (a) the control group after 2 weeks with severe destruction in the form of abscess formation (AB) and spicules of bone (S) without any osteocytic lacunae, (b) the 2.5 mg group after 2 weeks showing minute hard tissue formation (NB) in the vicinity of the tooth (T), (c) the 5 mg group after 2 weeks showing increase in newbone formation(NB) marked with red by Goldner trichrome, (d) the control group after 4 weeks with heavy necrotic tissues in between the widely open apical foramen. (Goldner Trichromex40), (e) the 2.5 mg group after 4 weeks. Note the newly formed bone (NB) with wide osteocytic lacunae and osteoblastic rimming (OB), (f)the 5mg group after 4 weeks showing the newly formed periapical bone (NB) (Masson-Goldner Trichromex100)

Osteopontin immunostained sections

Microscopic examination of osteopontin immunostained sections revealed diffuse osteopontin immunoexpression in the collagen fibers of the periapical area surrounding minimal bone formation in the control group after 2 and 4 weeks (fig. 3a,d). The 2.5 gm group after 2 weeks and 4 weeks showed little bone formation surrounded by osteopontin immunoexpressionin the collagen fibers of the periapical area which appeared more after 4 weeks. Osteopontin immunoexpression was expressed more in collagen fibers in close proximity to hard tissues (fig. 3b,e). The 5 mg group after 2 and 4weeks showed obvious hard tissue formation surrounded by strong osteopontin immunoexpressionin the collagen fibers of the periapical area especially those that are in close proximity to hard tissues which was more pronounced after 4 weeks(fig.3c,f)

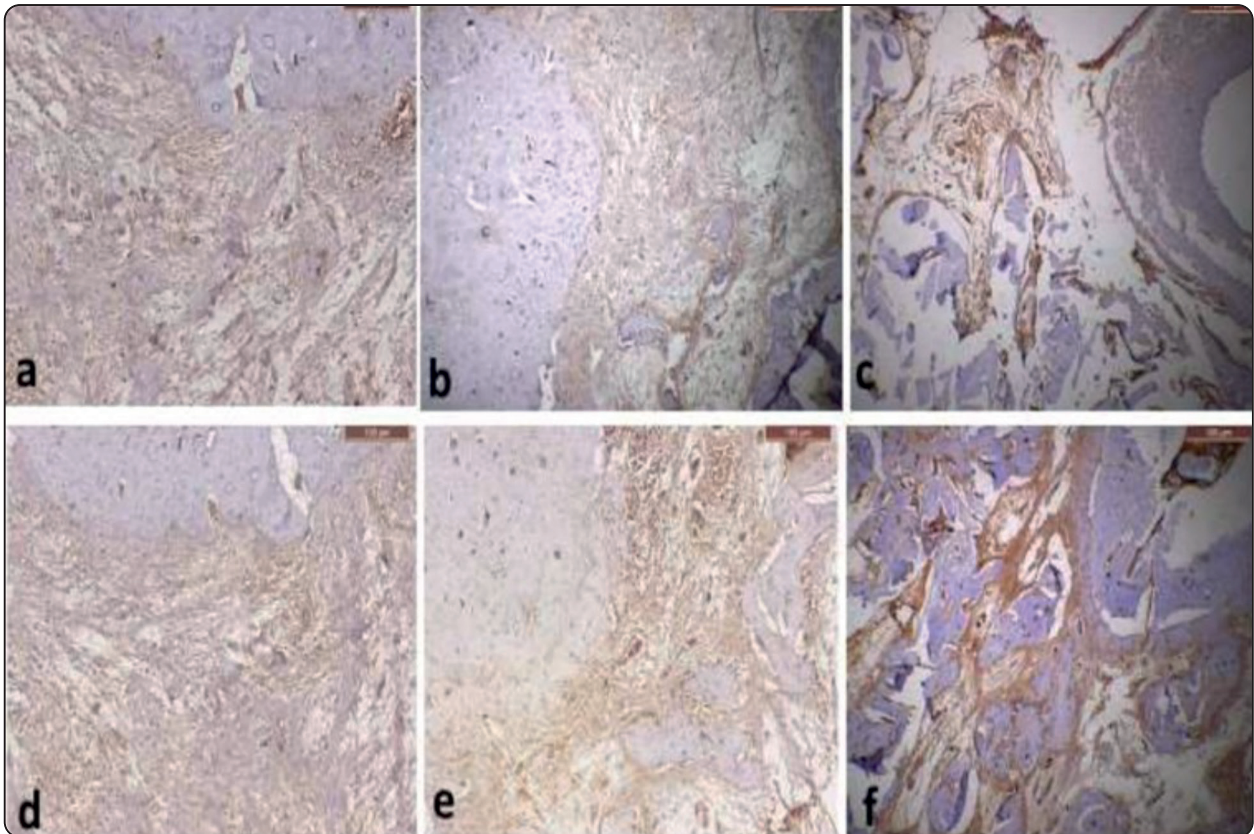

Fig. (3): Fig. 3: Photomicrograph of (a,d) the control group after 2 and 4 weeks showing diffuse osteopontin immunoexpression in the collagen fibers of the periapical area, (b e) the 2.5 mg group after 2 weeks and 4 weeks showing osteopontin immunoexpression accentuated in the stroma around hard tissue formation which appeared more after 4 weeks, (c,f) the 5 mg group after 2 and 4weeks showing obvious hard tissue formation surrounded by strong osteopontin immunoexpression in the stroma of the periapical area and accentuated in collagen fibers around the hard tissues, (OsteopontinX 100)

Statistical analysis

The area percent of Masson-Goldner trichrome expression was enhanced by time. After 4 weeks, the area percent for the control, 2.5mg and 5mg group was greater than after 2 weeks, but the difference reached significance in the 5mg group (table 1).

TABLE (1) Comparing the area percent of Masson-Goldner trichrome expression after 2 and 4 weeks in the control, 2.5mg and 5mg groups. (Unpaired student t-test)

Duration Drug dose	2 weeks	4 weeks	P value
Control group	3.51±1.89	7.89±6.78	P=0.2
2.5mg group	10.89±5.49	20.11±10.38	P=0.12
5mg group	13.11±3.15	38.48±8.52	P=0.0002*

In addition, the medication dosage increased the percentage of area expressing the Masson-Goldner trichrome. After 2 weeks, there was no statistically significant difference between the 5mg and 2.5mg groups in terms of area percent. 4 weeks later, the 5mg group had a considerably higher area percent than the 2mg group (table 2).

TABLE (2) Comparing the area percent of Masson-Goldner trichrome expression in the control, 2.5mg and 5mg group after 2 and 4 weeks.(Unpaired student t test)

Drug dose Duration	2.5mg group	5mg group	P value
2 weeks	10.89±5.49	13.11±3.15	P=0.46
	Control group	**2.5mg group**	**P value**
	3.51±1.89	10.89±5.49	P=0.02*
	Control group	**5mg group**	**P value**
	3.51±1.89	13.11±3.15	P=0.0004*
4 weeks	**2.5mg group**	**5mg group**	**P value**
	20.11±10.38	38.48±8.52	P=0.02*
	Control group	**2.5mg group**	**P value**
	7.89±6.78	20.11±10.38	P=0.06
	Control group	**5mg group**	**P value**
	7.89±6.78	38.48±8.52	P=0.0002*

The area percent of osteopontin immunoexpression was enhanced by time. After 4 weeks, the area percent for the control, 2.5mg and 5mg group was significantly greater than after 2 weeks (table 3).

TABLE (3) Comparing the area percent of osteopontin immunoexpression after 2 and 4 weeks in the control, 2.5mg and 5mg groups. (Unpaired student t test)

Duration Drug dose	2 weeks	4 weeks	P value
Control group	13.04±1.82	21.14±1.92	P<0..001*
2.5mg group	25.97±0.27	32.31±1.47	P<0..001*
5mg group	30.6±1.18	48.96±2.69	P<0..001*

Additionally, the medication dosage increased osteopontin immunoexpression per unit area. The 5mg group had a considerably higher area percent of osteopontin immunoexpression than the 2mg group after 2 weeks. Similarly, after 4 weeks, the 5mg group had considerably higher area percent of osteopontin immunoexpression compared to the 2mg group (table 4).

TABLE (4) Comparing the area percent of osteopontin immunoexpression in the control, 2.5mg and 5mg group after 2 and 4 weeks.(Unpaired student t test)

Duration Drug dose	2.5mg group	5mg group	P value
2 weeks	25.97±0.27	30.6±1.18	P<0..001*
	Control group	**2.5mg group**	**P value**
	13.04±1.82	25.97±0.27	P=0.0001*
	Control group	**5mg group**	**P value**
	13.04±1.82	30.6±1.18	P<0.0001*
4 weeks	**2.5mg group**	**5mg group**	**P value**
	32.31±1.47	48.96±2.69	P<0..001*
	Control group	**2.5mg group**	**P value**
	21.14±1.92	32.31±1.47	P<0.0001*
	Control group	**5mg group**	**P value**
	21.14±1.92	48.96±2.69	P<0.0001*

Furthermore,the area percent of Masson-Goldner trichrome and osteopontin expression in the 2.5mg group after 4 weeks was greater than the 5mg group after 2 weeks group, but the difference was not statistically significant for both stains (table 5).

TABLE (5): Comparing the area percent of Masson-Goldner trichrome and osteopontin immunoexpression in the 2.5mg after 4 weeks group versus the 5mg after 2 weeks group. (Unpaired student t test)

Group	2.5mg 4w group	5mg 2w group	P value
Area percent (Masson-Goldner trichrome)	20.11±10.38	13.11±3.15	P=0.2
Area percent (osteopontin)	32.31±1.47	30.6±1.18	P=0.08

DISCUSSION

Simvastatin, a widely accepted drug used primarily to lower the cholesterol levels has revealed increase in bone mineral density with long term systemic administration in humans [24]. As an added bonus, stem cells from dental pulp that were treated with simvastatin developed mineralized tissue more quickly [25].

In this work, simvastatin was used in concentration of 2.5 mg/ml similar to Wong and Rabie 2005 [26] study, and in concentration of 5 mg/ml similar to Bayuomi et al., 2020 [27] to compare the effect of both concentrations with each other and with the control as intracanal dressing on healing of induced periapical lesions in dogs. The drug was left in the canals for one week before obturation which is the time period during which new periapical collagen usually starts to be regenerated, and to give enough time to simvastatin to be in contact with the periapical tissues so as to be able to affect the periapical healing. Dogs were left after obturation for 2 and 4 weeks follow-up periods before sacrifice, at which detection of periapical healing area could be examined, similar to Anbinder et al. 2006 [28] and Ahmed et al. 2007 [29] studies.

Healing of bone was evaluated in this study by measuring the area percent for Masson-Goldner trichrome histochemical stain and osteopontin immunhistochemical stain. Bone histologists often utilise the Masson-Goldner trichrome staining technique because it facilitates both color-based and morphologically-based tissue classification[30].

Secreted osteopontin is a phosphorylated, acidic, calcium-binding glycoprotein that attracts macrophages, smooth muscle cells, endothelial cells, and glial cells. It may also bind to collagen I to engage the extracellular matrix. Staining for osteopontin was positive in the dense connective tissue around the scaffold fibres and in the osteoblasts close to the bone-connective tissue interface. It's obvious that osteopontin is connected to collagen fibres[30].

Osteopontin is expressed early in mesenchymal cell differentiation and is related to osteogenesis. At different stages of osteogenic differentiation in cultures of fetal rat calvarialcells, osteopontin was expressed by 60–98% of cells [31]. The bone matrix protein osteopontin has been shown to have a role in controlling bone mineralization. Osteopontin was evaluated in a rabbit model of a lengthy bone defect of crucial size in vivo, where it showed promise for promoting bone regeneration by induction of stem cell proliferation, osteogenesis, and enhancement of angiogenic characteristics [32].

In our study, the group treated with simvastatin showed more bone formation compared to the control group. This in accordance with Delan et al., 2021 [33] who found that the groups treated with simvastatin showed more bone formation in periodontal bony defects in rabbits due to its bone inducing effect.

In this study, the effect of simvastatin was enhanced by time. The area percent of Masson-Goldner trichrome stain after 4 weeks was greater than after 2 weeks for the control, 2.5mg and 5mg

group, however the difference reached significance only in the group taking a higher drug dose (5mg group).Similarly, the area percent of osteopontin immunoexpression after 4 weeks was greater than after 2 weeks and was significant for all groups (the control, 2.5mg and 5mg group).

The previous findings show that osteopontin immunoexpression is more indicative for bone formation than Masson-Goldner trichrome stain as osteopontin is expressed early during mesenchymal differentiation [31] in in association with collagen fibers before actual bone formation [30]. On the other hand, Masson-Goldner trichrome is a marker for new bone formation [30].That is why its expression was significantly higher after exposure to a higher drug dose for a longer time (5mg for 4 weeks) which was more effective in induction of new bone formation.

Moreover, the effect of simvastatin was enhanced by the dose of the drug. The area percent of Masson-Goldner trichrome expression was greater in the 5mg group compared to the 2.5mg group and the difference was significant after 4 weeks. In the same way, the area percent of osteopontin immunoexpression was significantly greater for the 5mg group compared to the 2mg group at both time intervals.

Similar results were observed by Dang et al., 2021 [34] who discovered that simvastatin, when applied topically, induces intramembranous ossification by increasing expression of BMP-2. Bone repair after simvastatin treatment in Rhesus monkeys with calvarial abnormalities was shown to be equivalent to that after autograft. Simvastatin doses of 50, 100, and 200 milligrammes were employed, with effects measured 8 and 16 weeks later. Improving results were shown when administration of the medicine was prolonged and dosage was increased

More bone was found at higher drug doses of simvastatin (1-10mg kg1 d1), while the medicine was effective at increasing bone production and trabecular bone at lower drug doses[16,35]. Simvastatin-loaded porous titanium oxide surface may be useful in low-quality bone because it speeds up osseointegration and peri-implant bone development. The simvastatin-loaded groups had greater peri-implant bone volume and mineral apposition rate at 2 and 4 weeks compared to the control group. Even more impressive, the results improved after 4 weeks [36].

This evidence revealed that simvastatin accelerates the production of new bone and that this effect became stronger with time. This is due to the fact that it has the ability to initiate the expression of growth factors that control angiogenic processes, bone cell differentiation, and bone formation (osteogenesis) at an early stage [26].In addition to lowering cholesterol, simvastatin may help bones recover by reducing the number of bone-resorbing osteoclasts and increasing the number of bone-building osteoblasts at fracture sites [37]. This may be also due to its capacity to downregulate interleukin-1beta and prostaglandin E2 production [38]. This conclusion was consistent with previous studies [21, 26,37], however it differed from studies done by Anbinder et al. 2006 [28] and Ma et al. 2008 [39], which is due to different mode of application of simvastatin, where it was administered orally and subcutaneously in the Anbinder et al. 2006 [28] study, and orally in the Ma et al. 2008 [39] study.

Comparing the area percent of Goldner trichrome and osteopontin immunoexpression in the 2.5mg after 4 weeks group versus the 5mg after 2 weeks group,it was found that the effect of low dose of drug for long period was higher but not statistically significant than high doses of the drug for a shorter period. This data is beneficial for further clinical considerations in humans to avoid drug overdose.

Statistical analysis in the present work was emphasized by microscopic examination of Masson-Goldner trichrome stained sections. Microscopic examination after 2 weeks showed spicules of bone without any osteocytic lacunae in the control group, while the 2.5 gm group showed minute hard tissue

formation which was relatively increased in the 5mg group. After 4 weeks, the 2.5mg group revealed newly formed bone with wide osteocytic lacunae and osteoblastic rimming which was more pronounced in the 5mg group. These results are consistent study [40] that reported that simvastatin possibly can improve the quality of osteogenesis during healing of dental sockets as the percentage of vital bone was more in the groups treated with simvastatin. However, further studies were recommended by the authors to have definite results.Another research [41] found that following tooth extraction in male Wistar rats, the newly created bone island was bigger and the bone formation rate and quality were better than in the control group at 2, 4, 8, and 12 weeks, but worse at 1 week. The results suggested that simvastatin, applied locally, would promote healthy bone growth in the extraction socket, so preserving the remaining alveolar bone.

Microscopic examination of osteopontin immunostained sections revealed diffuse osteopontin immunoexpression in the collagen fibers of the periapical areasurrounding minimal bone formation in the control group after 2 and 4 weeks. The 2.5 gm group after 2 weeks and 4 weeks showed little bone formation surrounded byosteopontin immunoexpression which appeared more after 4 weeks. Osteopontin immunoexpression was expressed more in collagen fibers surrounding hard tissues. The 5mg group after 2 and 4 weeks showed obvious hard tissue formation surrounded by strong osteopontin immunoexpression in the collagen fibers of the periapical area especially at the bone-connective tissue interphase which was more pronounced after 4 weeks. This finding is in accordance with a study (30)that illustrated that positive osteopontin staining was evident at the firm connective tissue areas around the scaffold fibers as well as at the osteoblasts near the bone-connective tissue border. They authors added that osteopontin was clearly associated within the collagen fibers

As a conclusion, the present study showed a beneficial effect of simvastatin on the healing of apical periodontitis. However, it must be emphasized that a complete root canal therapy to eliminate the pathogens is most critical in the treatment of apical periodontitis. Statins may serve to enhance the therapeutic effect but not to replace standard root canal treatment. Simvastatin as an intracanal medication can promote periodontal bone repair and accelerate healing.

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