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THE PROTECTIVE EFFECT OF LACTOFERRIN ON THE ALVEOLAR BONE LOSS INDUCED BY CARBAMAZEPINE IN RATS

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ABSTRACT

Introduction: Carbamazepine is one of the widely used anti-epileptic medications associated with elevated fracture rates. Lactoferrin is a secretory glycoprotein with iron binding capacity and one of the transferrin family of proteins. It is naturally found in colostrum and milk in the highest concentrations. It is found to have protective anti-inflammatory, antioxidant, and antimicrobial actions.

Aim: This study was designed to evaluate the protective influence of lactoferrin on the alveolar bone damage mediated by carbamazepine in rats.

Materials and Methods: Twenty-four healthy female Wistar albino rats were randomly distributed into 3 groups, 8 rats each. Group I-control, rats were given a daily dose of saline. Group II- carbamazepine, rats received carbamazepine 75mg/kg/day orally for 5 weeks. Group III-carbamazepine and lactoferrin, rats received carbamazepine 75mg/kg/day in addition to lactoferrin 100 mg/kg/day orally for 5 weeks. After euthanasia, the mandibles of all rats were collected and prepared to be evaluated by light and scanning electron microscopy, histomorphometry, and energy-dispersive x-ray microanalysis.

Results: The alveolar bone in the carbamazepine group showed marked porosity and trabecular thinning in comparison to the control and lactoferrin groups demonstrated by both light and scanning electron microscopy. The bone volume ratio values were significantly higher in carbamazepine-lactoferrin and control groups than in the carbamazepine group. The percentage of calcium mineralization detected by Energy Dispersive X-ray spectroscopy in lactoferrin group was comparable to the control and significantly higher than carbamazepine group.

Conclusions: Lactoferrin supplementation could successfully reduce the porosity provoked by anti-epileptic medications in the alveolar bone.

KEYWORDS: Lactoferrin, alveolar bone loss, Carbamazepine, antiepileptic drugs (anticonvulsants), rats.

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INTRODUCTION

Epilepsy is a serious disorder affecting the brain that may provoke morbidity and premature mortality due to the associated seizures [1]. Epilepsy affects over 50 million people Worldwide [2]. In most cases, anti-seizure medications are considered the first line of treatment to control convulsions. Once correctly diagnosed, the initiation of drug treatment is recommended the earliest until long-term seizure freedom is reported [1].

Unfortunately, the utilization of antiepileptic drugs (AEDs) has been correlated with multiple neuropsychiatric side effects, metabolic and endocrinal disturbances, and teratogenicity[1]. Anticonvulsant medications have also been proven to affect bone health leading to secondary osteoporosis through the reduction of bone mineral density and bone mass[1,3]. Elderly patients, children, and postmenopausal women represent the high-risk groups[3,4]. Other risk factors include the duration and dosage of treatment, polytherapy, sedentary lifestyle, reduced sun exposure, smoking, and alcohol consumption[2,3]. An increased fracture risk of 2-6 folds has been reported in epileptic patients on anti-seizure drug regimens; regardless of the seizure-related fractures, which account for only 25-43% of cases[2,3].

Carbamazepine (CBZ) is one of the widely used anti-epileptic medications associated with elevated fracture rates[3]. CBZ is approved by the United States Food and Drug Administration (FDA) as a medication for epileptic seizures, trigeminal neuralgia, and bipolar disorder[5]. This drug is classified as one of the hepatic enzymes (cytochrome P450) inducers group of AEDs characterized by their potential adverse influence on bone even on monotherapy. Other hepatic enzyme inducers include oxcarbamazepine, valproic acid, phenytoin, phenobarbital, and primidone [4]. This group of inductor drugs could alter vitamin D metabolism, suppress calcium intestinal absorption, and increase the secretion of parathyroid hormone. Therefore, these drugs disrupt the extent of bone mineralization by the mobilization of calcium from the skeleton to correct hypocalcemia[3,4]. According to records of previous studies, CBZ significantly reduces calcium and vitamin D levels and increases alkaline phosphatase levels (bone turnover marker), which indicates bone destruction. CBZ also mediates osteoclastogenesis by elevating the receptor activator of nuclear factor kappa beta NF-κβ ligand (RANKL) [6]. Since the alveolar bone has a high turnover rate, it could be more severely affected by CBZ showing a significant reduction in bone density[7]. Therefore, measures should be taken to lessen the risk of fracture in epileptic patients. Calcium and vitamin D supplementations are recommended in high-risk individuals in addition to anti-osteoporotic drugs such as bisphosphonates[8].

However, the chronic administration of bisphosphonates is associated with the possible occurrence of osteonecrosis of the jaw and atypical femur fractures [9].

Lactoferrin is a secretory glycoprotein with iron binding capacity and one of the transferrin family of proteins. It is naturally found in colostrum and milk in the highest concentrations, saliva, tears, bile, gastrointestinal, bronchial, and nasal secretions in addition to vaginal mucus, seminal fluids, and urine[10,11]. Lactoferrin is also present in neutrophils' secondary granules that degranulate its content in case of inflammation or infection[11]. In 1939, lactoferrin was first isolated from bovine milk and since then it has been studied extensively and shown many physiological and biological roles[10]. Human and bovine lactoferrin (bLF) have been found to share a high degree of similarity up to 78%[11].

Generally, LF has proved to have protective anti-inflammatory, antioxidant, and antimicrobial actions. lactoferrin could boost the immune system, increase iron absorption and reduce the risk of infections [10, 11]. Moreover, due to its high affinity to iron, LF could improve hemoglobin and reduce the availability of iron for pathogens [12]. Lactoferrin

has also been introduced in several neuropathologic disorders such as Parkinson's, Alzheimer's disease, and multiple sclerosis; as well as in cancers like cervical, breast cancer, and leukemia [11]. Additionally, recent studies revealed that LF could fight coronavirus infection by reducing the proinflammatory mediators and preventing the cytokine storm [13]. Both the FDA and European Food Safety Authority have approved the safety of bovine lactoferrin dietary supplementation. The safety of recombinant human LF (rhLF, talalactoferin) form has also been demonstrated by studies, its use was safe for 15 days. However, further studies were recommended to test the effective applications of both bLF and rhLF [12].

The benefits of lactoferrin on bone have been also reported, LF stimulates bone formation by promoting osteoblasts proliferation and differentiation and inhibiting their apoptosis [14, 15]. It could also suppress bone resorption by increasing the expression of osteoprotegerin (OPG) and inhibiting RANKL[16]. It could also prevent bone damage by suppressing the oxidative stress induced by the inflammatory state [14].

Since no previous studies in the literature have searched the role of lactoferrin in preventing the bone porosity induced by antiseizure medications. The purpose of the presented experimental work was to explore the effectiveness of lactoferrin in the reduction of the loss induced by carbamazepine in the alveolar bone of rats.

MATERIAL AND METHODS

This experimental protocol was approved by the Scientific Research Ethics Committee at the Faculty of Dentistry, Alexandria University (International No: IORG 0008839) and the procedures conform to the ARRIVE guidelines [17]. The sample size was estimated to be 24 rats, 8 rats per group assuming a 95% confidence level and 90% study power. The required sample size was calculated based on the Larry Connors method, using the Med Calc statistical software ver. 18.11. A previous study carried out by Guo, et al., 2009, was used to predict the different measurements in our study [18]. The current study was conducted on Twenty-four healthy Female adult Wistar albino rats (age ±10 weeks, weight 160-180 grams). Animals suffering from any illness or wounds or that were included in previous studies or genetically modified were excluded. Animals were acclimatized for 2 weeks in the animal house of the Faculty of Medicine, Alexandria University before starting the experiment. Animals were kept in polypropylene cages, 4 rats per cage, under 12:12 hours day/night cycles, controlled noise and humidity and at a temperature (24±2°C) with free access to water and a standard diet. Rats were randomly distributed using computer-generated random lists into 3 groups: Group I- control (n=8), rats received a daily intragastric administration of saline. Group II- carbamazepine (CBZ) (n=8), rats received carbamazepine (Tegretol 200, Novartis Pharma, Cairo, Egypt) 75mg/kg/day orally dissolved in saline for 5 weeks. Group III- carbamazepine and lactoferrin (Pravotin 100mg, Hygint Pharmaceuticals, Alexandria, Egypt) (CBZ+LF) (n=8), rats received 75mg/kg/day in addition to lactoferrin 100 mg/kg/day orally for 5 weeks. Doses of CBZ and LF were identified according to previous studies[19, 20]. Daily repetitive doses were introduced using gastric feeding to reduce pain and distress; the activity of animals was checked regularly, mobility and the ability of animals to access food and water were used as humane endpoints. After 5 weeks all animals were euthanized by an overdose of intravenous pentobarbital sodium (Nembutal, Akorn, New York, USA). After death confirmation, the mandibles were dissected and divided into two halves. Randomly, samples of one side were prepared for histological evaluation by light microscopy and histomorphometry. Samples of the other side were sectioned buccolingually and prepared for Scanning electron microscopy and Energy dispersive X-ray spectroscopy.

Preparation for histological evaluation

After euthanasia, a total of 24 mandibular halves (n=8 per group) were fixed in 10% neutral buffered formalin solution for 2 days, decalcified, then dehydrated in ascending concentrations of ethanol, cleared in xylene, infiltrated then embedded in paraffin wax blocks. Sections 4-5μm thick were cut and then stained by Hematoxylin and Eosin stain (H&E) and observed by light microscope (Optika, B-290 series, Ponteranica, Italy) to examine the bony structure of the alveolar process. Photomicrographs X100 and X400 were captured using a digital camera (Optika, C-B10, Ponteranica, Italy) coupled to the microscope. The structure of the alveolar bone was observed to detect histological signs of bone formation and resorption [21].

Histomorphometric analysis

Computer-assisted histomorphometry was performed by a single blinded examiner to measure the alveolar bone volume per tissue volume (bv/tv) on photomicrographs X100 of the H&E-stained sections at standardized depth using software Image J version 1.53K RRID: SCR_003070 (NIH, USA). Overall 24 measurements were taken from all samples, 1 average measure (average of 3 measures at apical, inter-radicular, and cervical regions) for each specimen (n=8 per group), and no animals were excluded [18].

Energy Dispersive X-ray (EDX) spectroscopy

Energy Dispersive X-ray microanalysis was employed for the elemental and chemical characterization of samples (n=8 per group) before preparation for scanning electron microscopy. The percentages of calcium (Ca) and phosphorus (P) in the alveolar bone were measured in all groups [22].

Preparation for scanning electron microscopy

After dissection, specimens (n=8 per group) were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH=7.2, sectioned buccolingually carefully with fresh sharp blades, and cleaned under a stereomicroscope. After fixation and EDX, samples were rinsed in 0.1 M phosphate buffer three times for 10 minutes and dehydrated by passing through a succession of 50%, 70%, and 95% ethyl alcohol for 10 minutes each followed by two changes of absolute alcohol for one hour each. The specimens were dried afterward by using the air-drying method in a vacuum desiccator in which air was evacuated by a rotator pump. The process was continued till the specimens were completely dried as evidenced by their color changing to white. The samples were mounted using silver paint on the specimen holder and coated with gold using a sputter coater [23]. Finally, samples were examined by scanning electron microscope (JEOL, JSM-200 IT, USA) to study the trabecular ultrastructure and extent of porosity of the alveolar bone[22]. Scalloping demarcates areas of resorption, white projections indicate sites of mineralization; surface smoothness is a mark of quiescence [24].

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). For continuous data, they were tested for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, and standard deviation for normally distributed quantitative variables. One-way ANOVA test was used for comparing the three studied groups and followed by Post Hoc test (Tukey) for pairwise comparisons. Significance of the obtained results was judged at the 5% level.

RESULTS

Histological evaluation

Observation of the alveolar bone by light microscope in the control group revealed normal architecture from the cervical (Fig. 1a,b) to the apical areas (Fig. 2a,b) as well as in the inter-radicular zone (Fig. 3a,b). A regular bone surface

lined by a continuous layer of flat to plump osteoblasts cells was revealed. Dense bone harboring osteocytes within lacunae of normal size and distribution. Incremental lines were also noted indicating active remodeling of bone. In the (CBZ) group, the alveolar bony structure showed signs of thinning and resorption illustrated by the irregular scalloped bone surfaces (Fig. 1c,2c,3c). Widened and empty osteocytes lacunae were also noticed (Fig. 1d,2d). Plump osteoblasts were scattered showing discontinuous arrangement and were seldomly observed (Fig. 3d). The (CBZ+LF) group showed an enhanced bony structure, and the surface showed a more regular outline (Fig. 1e,2e,3e). The resorption spots were followed by active deposition of new bone represented by organic matrix (osteoid) well-demarcated from mature bone by reversal lines (Fig. 1f,3f). No empty osteocytic lacunae were also observed, however, lacunar widening was noticeable (Fig. 2f).

Histomorphometric analysis

As shown in Table 1 and Figure 4 the mean bv/tv in the CBZ group was significantly lower than both the control and group III (CBZ+LF) (p1, p3 <0.001). No significant difference was shown in the mean bv/tv between CBZ+LF and the control group (p2=0.826).

Energy Dispersive X-ray (EDX) spectroscopy

As shown in Table 2 and Figure 5 the mean percentage of bone calcium in the CBZ group was significantly lower than both the control and group III (CBZ+LF) (p1, p3 <0.001). The mean percentage of phosphorus was also the lowest in CBZ, however, a statistically significant difference was only detected between the CBZ and CBZ+LF group (p3=0.003). No significant difference was detected between CBM+LF and the control group concerning the percentages of calcium and phosphorus (p2=0.496 and 0.061 respectively).

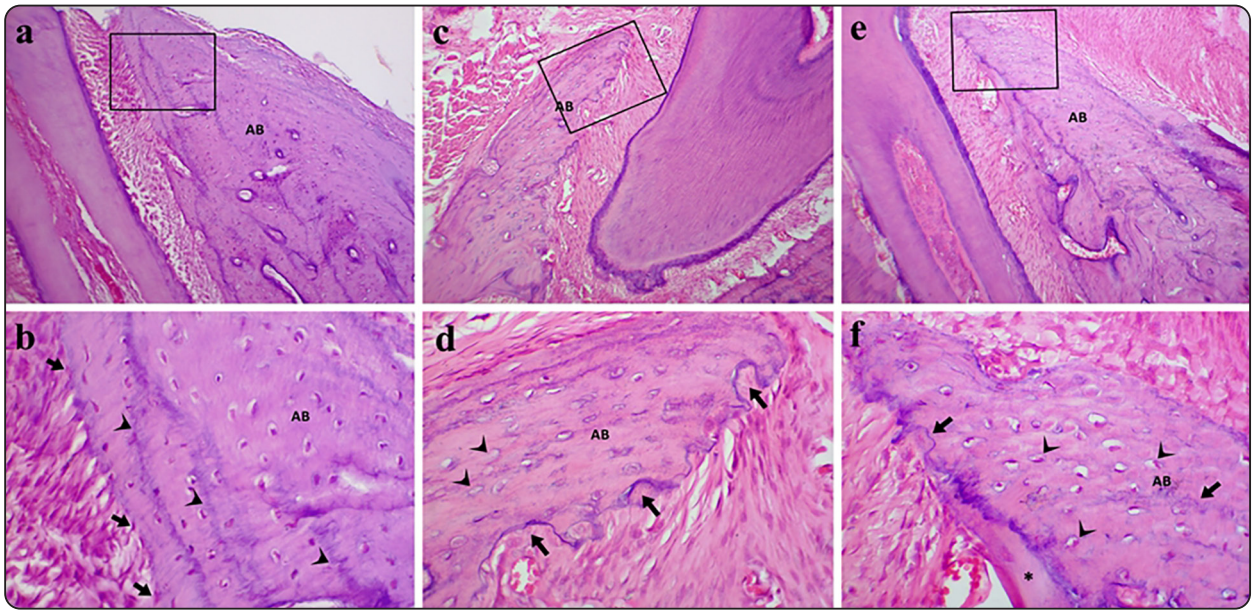

Fig. (1) Light photomicrographs of the alveolar bone at the cervical and middle parts in (a,b) Control group showing a regular bone surface lined by flat to plump osteoblasts cells (arrows), dense bone with incremental lines (arrowheads), and normal size and distribution of osteocytes lacunae (c,d) Carbamazepine group showing irregular scalloped bony surfaces (arrows) and empty osteocytes lacunae (arrowheads) (e,f) Lactoferrin group showing restoration of the surface regularity, organic matrix (Asterisk) formed adjacent to reversal lines (arrows), and osteocytic lacunae of normal size (arrowheads), AB: Alveolar bone [a,c,eX100 ; b,d,f X400]

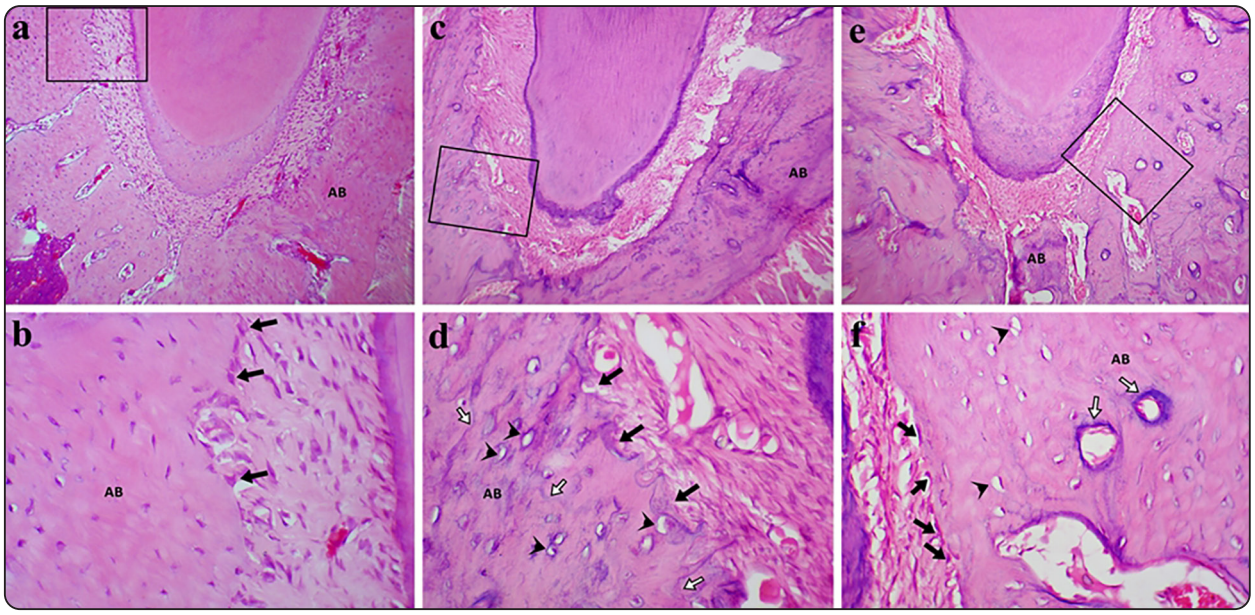

Fig. (2) Light photomicrographs of the alveolar bone at the apical area in (a,b) Control group showing smooth outline and plump osteoblasts (arrows) (c,d) Carbamazepine group showing a punched out appearance formed of Howship's concavities (black arrows), empty osteocytic lacunae (arrowheads) and multiple reversal lines (white arrows) (e,f) Lactoferrin group showing a regular bony outline with flat bone lining cells (black arrows), note wide osteocytic lacunae (arrowheads) and haversian canals of compact bone (white arrows), AB: Alveolar bone [a,c,eX100; b,d,f X400]

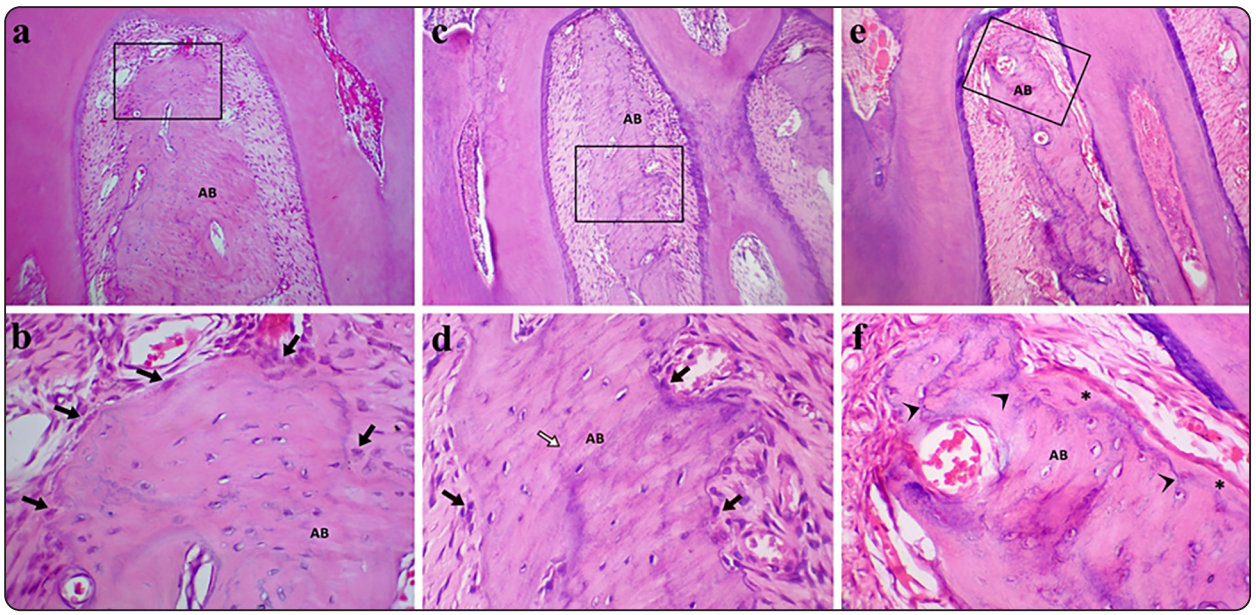

Fig. (3) Light photomicrographs of the alveolar bone at the inter-radicular area in (a,b) Control group showing bone surface with continuous arrangement of osteoblasts (arrows (c,d) Carbamazepine group showing multiple concavities lined by multiple osteoblasts (black arrows), note reversal lines (white arrows) (e,f) Lactoferrin group showing layers of osteoid lighter in color (asterisk) well demarcated from mature bone by deeply stained reversal lines (arrowheads) AB: Alveolar bone [a,c,eX100; b,d,f X400]

TABLE (1) Comparison between the three studied groups according to Bone volume per tissue volume (bv/tv)

	Control (n = 8)	CBZ (n = 8)	CBZ+LF (n = 8)	F	p
BV/TV					
Min. – Max.	0.387 – 0.435	0.215 – 0.368	0.351 – 0.454	24.897*	<0.001*
Mean ± SD.	0.411[a] ± 0.019	0.280[b] ± 0.061	0.399[a] ± 0.030		
p_1<0.001* , p_2=0.826 , p_3<0.00[1]*					

SD: Standard deviation
F: F for One way ANOVA test, pairwise comparison by Post Hoc Test (Tukey)
p: p value for comparing the three studied groups ***p1: p value for comparing between Control and CBZ***
p2: p value for comparing between Control and CBZ+LF ***p3: p value for comparing between CBZ and CBZ+LF***
****: Statistically significant at p < 0.05*** ***Means with Common letters are not significant***

TABLE (2) Comparison between the three studied groups according to percentages of calcium and phosphorus

	Control (n = 8)	CBZ (n = 8)	CBZ+LF (n = 8)	F	p
Calcium					
Min. – Max.	23.85 – 49.65	7.39 – 23.50	23.0 – 41.92	15.759*	<0.001*
Mean ± SD.	34.79[a] ± 9.17	15.66[b] ± 5.46	30.68[a] ± 6.37		
p_1<0.001* , p_2=0.496 , p_3=0.001*					
Phosphorus					
Min. – Max.	4.91 – 16.38	6.07 – 12.22	13.72 – 17.27	7.262*	0.004*
Mean ± SD.	11.20[ab] ± 4.61	9.19[b] ± 2.16	14.86[a] ± 1.19		
p_1=0.393 , p_2=0.061 , p_3=0.00*					

SD: Standard deviation
F: F for One way ANOVA test, pairwise comparison by Post Hoc Test (Tukey)
p: p value for comparing the three studied groups ***p1: p value for comparing between Control and CBZ***
p2: p value for comparing between Control and CBZ+LF ***p3: p value for comparing between CBZ and CBZ+LF***
****: Statistically significant at p <0.05***
Means with Common letters are not significant (i.e. Means with Different letters are significant)

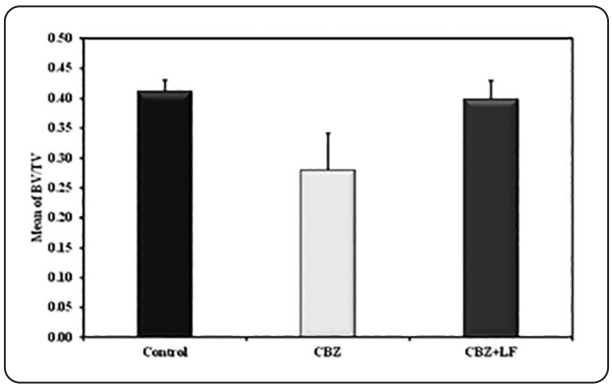

Fig. (4) Bone volume per tissue volume ratio among groups

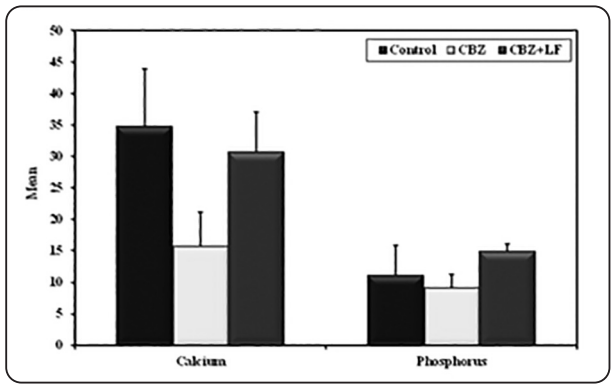

Fig. (5) Percentages of calcium and phosphorus in alveolar bone among groups

Scanning electron microscopy

Examination of the alveolar bone internal architecture in the control group by scanning electron microscope revealed thick trabeculae of bone with a smooth surface and normal-sized marrow cavities (Fig. 6a,b). The (CBZ) group revealed marked porosity, widening of marrow cavities (Fig. 7a,b), and extensive thinning of bone trabeculae represented by leaflike structures and areas of demineralized collagen fibers (Fig.7c,d). The (CBZ+LF) group showed enhanced bony structure with reduced porosity, generally, the trabeculae were notably thicker and the marrow cavities were narrower, however, some areas still showed trabecular thinning (Fig. 8a,b). The bony surface was generally smooth interrupted by areas of irregularities and roughness (Fig. 8c,d).

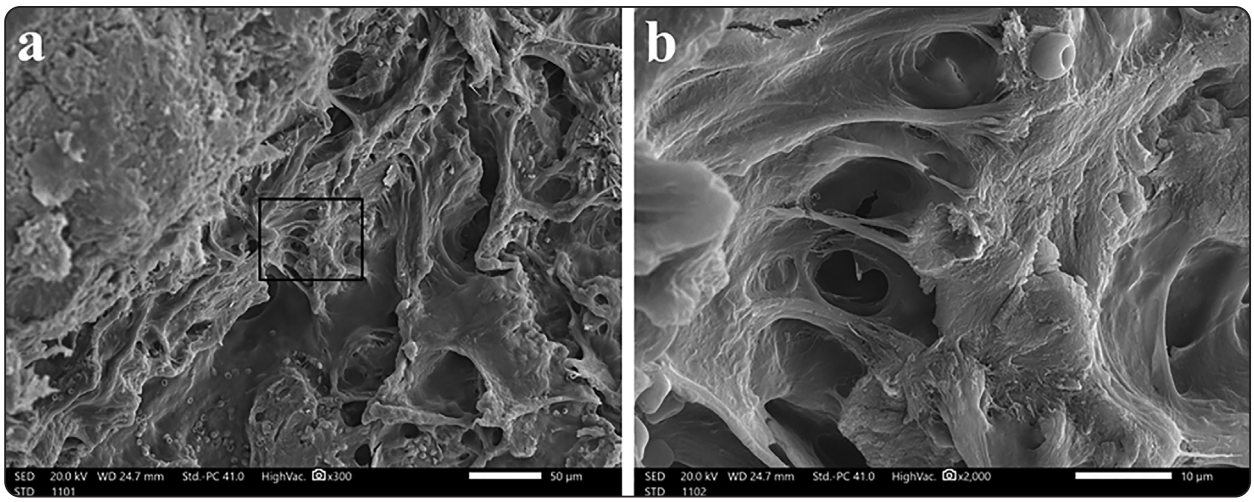

Fig. (6) Scanning electron photomicrographs of the ultrastructure of bone in the control group (a,b) showing thick trabeculae of bone with a smooth surface and normal-sized marrow cavities (X300, X2000 respectively)

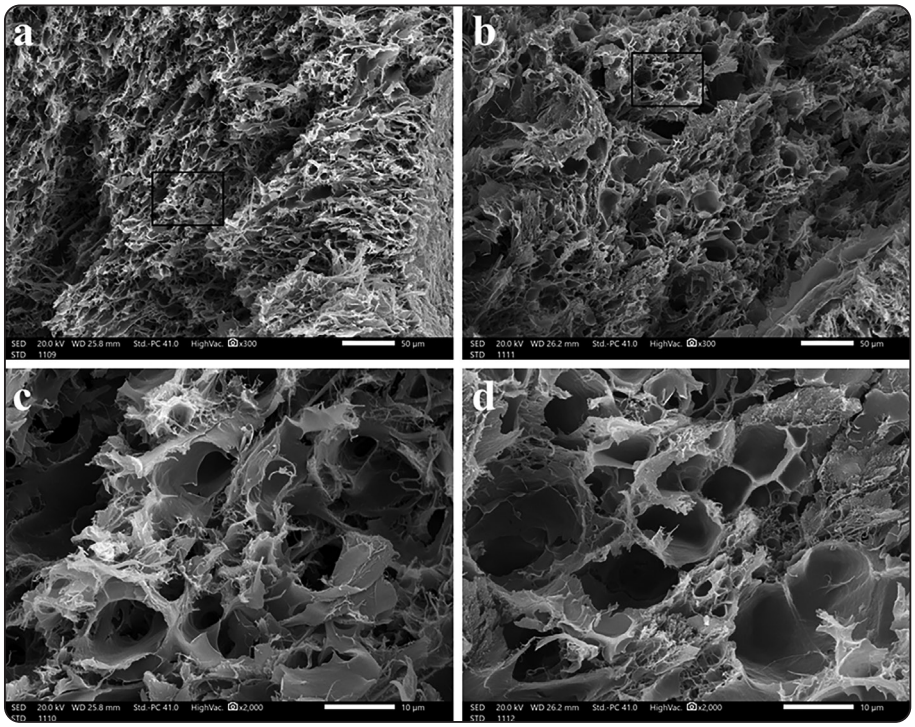

Fig. (7) Scanning electron photomicrographs of the ultrastructure of bone in the (CBZ) group (a,b) showing marked porosity, widening of marrow cavities(X300), (c,d) showing extensive thinning of bone trabeculae in a leaflike pattern, note areas of demineralized collagen fibers (X2000)

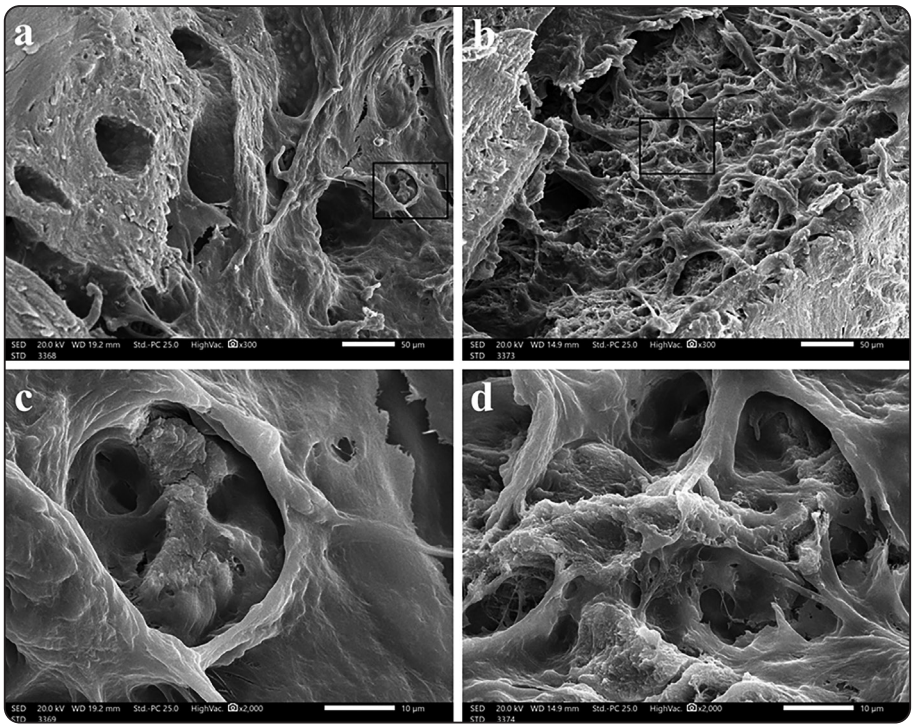

Fig. (8) Scanning electron photomicrographs of the ultrastructure of bone in the (CBZ+LF) group (a) showing enhanced bony structure with reduced porosity, the trabeculae were notably thicker, and the marrow cavities were narrower (b) persistent areas of trabecular thinning (X300), (c,d) smooth bony surface interrupted by areas of irregularities and roughness (X2000)

DISCUSSION

Epilepsy is a common neuropathological disorder that recommends the chronic or lifelong usage of anticonvulsants. One of the most serious adverse outcomes of the use of anti-seizure medications is the development of osteoporosis [2]. Carbamazepine is one of the widely used antiepileptic drugs recognized to alter bone health [6]. Interestingly, CBZ has been detected in human bone upon most mortem analysis in a case study accomplished by Fernández-López et al.[25], which explains its direct impact on the skeleton. Lactoferrin is an immunomodulator glycoprotein capable of suppressing reactive oxygen species and reducing inflammation. Due to its direct effect on cells, lactoferrin has been introduced in bone regenerative procedures and to promote bone health [14].

Therefore, the current study was designed to detect the role of lactoferrin in reducing the bone damage induced by the anti-seizure drug carbamazepine in rats. Animal models were selected in the study design to eliminate the effects of genetics, lifestyle, and individual differences in humans [26]. Rats are the most common animal models for osteoporosis, moreover, female rats were chosen because the female sex is considered one of the risk factors for the occurrence of osteoporosis [2].

The current results reflected the resorption of the alveolar bone upon the administration of the anticonvulsant drug CBZ for 5 weeks. This bone loss was manifested on the light microscopic level by the irregularity of bone surfaces and the abundance of bone-resorbing cells. In addition to the marked thickening and porosity revealed by electron microscopy and the significant reduction of bone surface area ratio. These findings agreed with Akhoundi et al.[7], who reported a significant reduction of alveolar bone density following CBZ treatment in rats, however no significant change in osteoclast number was detected. Moreover, Kanda et al. [27] also reported that other hepatic enzymes inducers like phenytoin significantly reduced bone formation parameters, bv/tv, trabecular thickness, and number in proximal tibia metaphysis of mice. Phenytoin also increased signs of bone formation

and separation between trabeculae [27]. Similarly, Güler et al.[28] revealed that intrauterine exposure to CBZ caused a significant dose-dependent reduction of femoral bone ossification. Histological evaluation by light microscope also showed smaller and fewer bony spicules. Scanning electron microscopy also showed thinning of the mineralized cartilaginous matrix [28].

On the contrary, Diemar et al.[29] did not report any significant changes in the trabecular thickness and separation or the bv/tv fraction, in the CBZ group at 60mg/kg, only the compact bone porosity was increased. However, the same study showed that Valproic acid (one of the enzymes inducers group of AEDs) induced a significant reduction in bv/tv in the femur and tibia [29].

The findings of the present research showed that lactoferrin counteracted the effect of CBZ through the preservation of the alveolar bony structure. The bone surfaces showed signs of new bone formation by light microscope. Thicker, less porous bone trabeculae were evident by electron microscopic examination in addition to a significant increase in bone volume ratio compared to the untreated (CBZ) group. The alveolar bone content of calcium and phosphorus in the CBZ+LF group was significantly higher than in the CBZ group and values were comparable to the control group. These findings agreed with Chen et al.[30] who described the role of bovine lactoferrin gavage for 14 days in improving the alveolar bone density in molars affected by periodontitis. The anti-inflammatory action of LF could be explained by the suppression of pro-inflammatory mediators IL-1 and TNF-α, and the initiation of anti-inflammatory cytokines, IL-10 and IL-4 to restore their normal balance (30). Likewise, the study of Guo et. al.[18] demonstrated that lactoferrin prevented vertebral and tibial trabecular bone loss caused by ovariectomy in rats. Moreover, LF was capable to restore the connectivity of bone segments and to prevent the reduction of bv/tv, trabecular thickness, and number in ovariectomized rats [18]. Cheng et. al.[31]also revealed that a low daily dose of LF as 10mg/kg preserved the bone volume/tissue volume ratio in newly formed bone during the rapid palatal expansion of the mid-palatal suture. However, the same investigation did not demonstrate the inhibition of osteoclastic bone resorption by LF, furthermore, the osteoclastogenesis process was not affected [31]. The study of Xiao et al. [32] also demonstrated that LF enhanced the migration and proliferation of osteoblasts cells, which could stimulate bone formation in the mid-palatal suture expansion. The levels of bone formation markers such as osteocalcin, and Collagen type I were distinctly elevated after treatment with LF (100 mg/kg and 1 g/kg) dose-dependently [32]. Additionally, Li et. al. [33] reported the regeneration of thicker new bone in the bony gap during distraction osteogenesis in rabbits' tibia upon the oral intake of bovine lactoferrin.

Fan et. al.[16] also stated that LF elevated the femoral bone Ca and P content while preserving the Ca/P ratio constant in ovariectomized mice as well as the bv/tv ratio. The in-vitro part of the study performed by Lee et al.[34] showed that lactoferrin was able to improve the osteogenic differentiation potential of the human adipose-derived stem cells with greater gene expression of osteopontin, osteocalcin, and Runt-related transcription factor 2 (RUNX2). In vivo implantation within mouse calvarial defects, LF demonstrated six times greater new bone formation than the results of other groups[34].

The antiresorptive effects of LF are due to its capacity to decrease the expression level of RANKL and increase that of OPG [16, 33]. The osteogenic effects of lactoferrin could also be explained by its documented capability to increase alkaline phosphatase activity (a marker of bone formation), in a concentration-dependent manner and directly induce osteoblastic autophagy essential for cellular homeostasis [35].

Lactoferrin proved to successfully reduce the porosity provoked by carbamazepine in the alveolar

bone of rat models. However, further clinical studies are recommended to investigate the incorporation of lactoferrin supplementations in the prevention of osteoporotic changes mediated by anti-epileptic medications.

CONCLUSION

We found that CS/nHA scaffolds can act as osteoconductive material aiding in bone regeneration. Moreover, upon application, it might be used to counteract the effect of osteoporosis. Therefore, it might be helpful for future surgical procedures during dental implantation in patients with osteoporosis.

Competing interests

The author has no competing interests to declare.

Funding

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Ethics approval

This research protocol received the approval of the Scientific Research Ethics Committee at the Faculty of Dentistry, Alexandria University (International No: IORG 0008839). Ethical approval is found in the supplementary information (Online resource 1).

Informed consent

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

HF and SH equally contributed to conceptualization, methodology, validation, data curation, investigation, resources, writing—original -original draft preparation, visualization, and writing—reviewing and editing.

ARRIVE guidelines

This study has been carried out in compliance with the ARRIVE guidelines for reporting in-vivo animal research experiments. The ARRIVE checklist available upon request

RECOMMENDATIONS

According to the results of the current experiment, we recommend the following:

1. The use of different intervals to evaluate the role of CBZ on bone structure.
2. Different doses of Lactoferrin should be administered to evaluate its protective role on bone structure.
3. More studies are recommended to study the effect CBZ and Lactoferrin on different dental and paradental tissues.

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