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RESEARCH ARTICLE

Assessment of the Effect of Local Application of Exogenous Melatonin on Bone Healing Process

Muhanad Tareq Jehad*, Ban A. Ghani

College of Dentistry/Baghdad University/Iraq.

***Corresponding Author Email:** *dr_muhanad1980@yahoo.com*

Abstract

Bone defects have become universal health care difficulty. Bone regeneration involves the differentiation of new cells and the formation of new bone tissues that results in an overall increase in volume of new skeletal tissues. Medical treatments for bone defects have been largely focused on replacing the lost bone with bone grafts materials. Aim of the study: is to evaluate the effect of local application of melatonin on healing of induced bone defect by histological and histomorphometric analysis. Materials and methods: Twenty four adult male rats divided into control and melatonin groups(8 animals) for each healing periods (1,2, and 4 weeks).Two holes induced in each rat femur (one hole in right for melatonin application and one in left femur bones). Results: Histological findings indicated that bone defects in the melatonin group showed early bone formation, mineralization and maturation as compared to control ones. Mean values of studied (bone trabecular area and number) were higher in melatonin group and values of (bone marrow area) decreased in 2 and 4 weeks durations. Osteoblasts, osteocytes mean values were higher in melatonin group, osteoclasts highest values shown in 2weeks in control group. Conclusion: The application of melatonin was effective in bone healing process by enhancement of earlier bone formation.

Keywords: *Bone defect, Melatonin, Histomorphometric analysis.*

Introduction

Bone has a substantial capacity for repair and regeneration in response to injury or surgical treatment from which involves a complex integration of cells, growth factors, and the extracellular matrix [1]. Bone is a dynamic tissue undergoing remodeling throughout life, and this remodeling requires a balance between deposition of new bone by osteoblasts and resorption of old bone by osteoclasts [2].

Bone remodeling requires the interaction between multiple bone cells (osteoblasts/ osteoclasts/ osteocytes) to renew, maintain, or adjust bone strength and/or mineral homeostasis in response to changing environmental influences.

There are four distinct phases to this process: activation, resorption, reversal, and formation with resorption and formation taking place via osteoclasts and osteoblasts, respectively [3]. Melatonin is an endogenous hormone rhythmically produced in the pineal gland under the control of the suprachiasmatic nucleus (SCN) and the light/dark cycle. This indole amine plays an important role in many physiological processes. The investigation and applications of melatonin in the hard tissues bone and tooth have received great attention. Melatonin has been investigated relative to bone remodeling, Osseo integration of dental implants and dentine formation [4].

Materials and Methods

Twenty four adult male rats (*Rattus norvegicus*) weighing (250-350 g), age (7-8months) were used in this study. The animals were randomly divided into control and melatonin group (8 animals) for each healing periods (1, 2, and 4 weeks). Two holes of about 2.5 mm in depth 2.5 mm in diameter (depth and diameter have been checked by using vernia) induced in each rat femur [5].

The application of materials was done as follows:

Control Group (C)

Bone defect left to heal spontaneously (left femur).

Melatonin Group (M)

Local application of melatonin (0.5 mg dissolved in 0.1 ml propylene glycol was performed (right femur) [6].

Scarification of all animals was done for the aforementioned healing periods. Specimens were prepared for histological and histomorphometric analysis .Histomorphometric measurements were determined using specific software (image J program) which analyzes the microscopic images [6].

Results

Histological findings of the control group (one week duration) illustrates deposition of osteoid bone by osteoblasts that appeared at its peripheries, osteocytes occupying their lacunae trapped in bone matrix (Figure 1). Melatonin group (first week duration shows spicules of new bone enclosing large number of irrregullarly distributed ostecytes ,formative osteoblasts seen at edges of bone (Figure 2). In control group (two weeks duration) shows bone trabeculae enclosing areas of marrow tissue (Figure 3).Melatonin group (two weeks duration) shows deposition of bone trabeculae that replace areas of melatonin material, the osteoblasts are seen at peripheries of the bone ,osteocytes seen trapped in bone and reversal line between old and new boneis illustrated, also progenitor cells are noticed (Figure 4).

Control group (four weeks duration) shows deposition of mature bone that fills the operating site, osteoblasts positioned at peripheriesand osteocytes (Figure 5). Melatonin group (four week's duration) illustrates mature dense bone in defect site ,osteoblasts seen at rims of bone and osteocytes. (Figure 6).

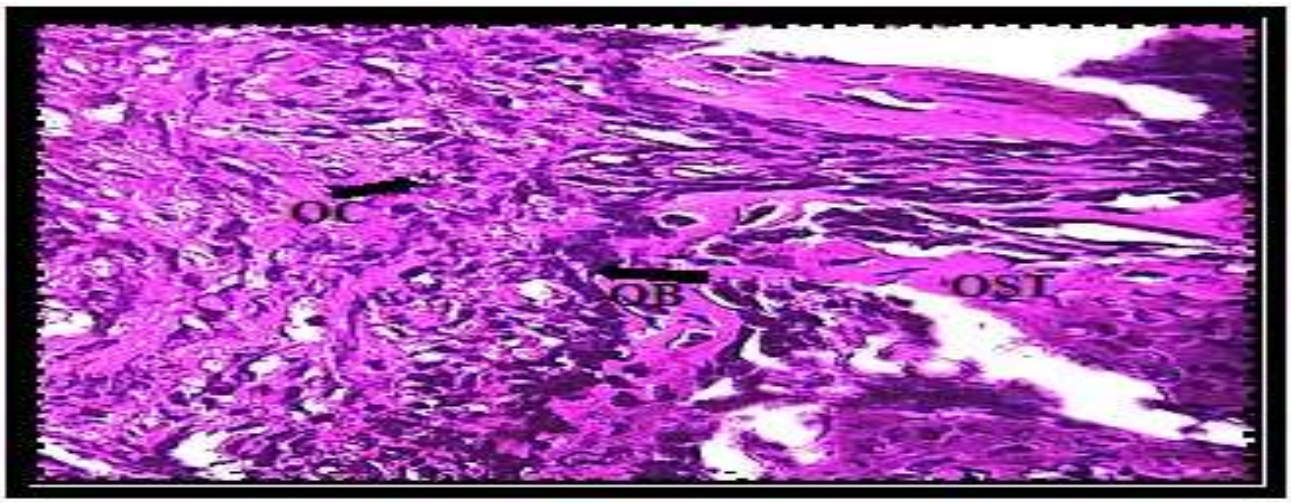

Figure 1: View of defect site of control group of 1week duration shows osteoid bone (OST), osteoblasts(OB)and osteocytes(OC) .H&EX40

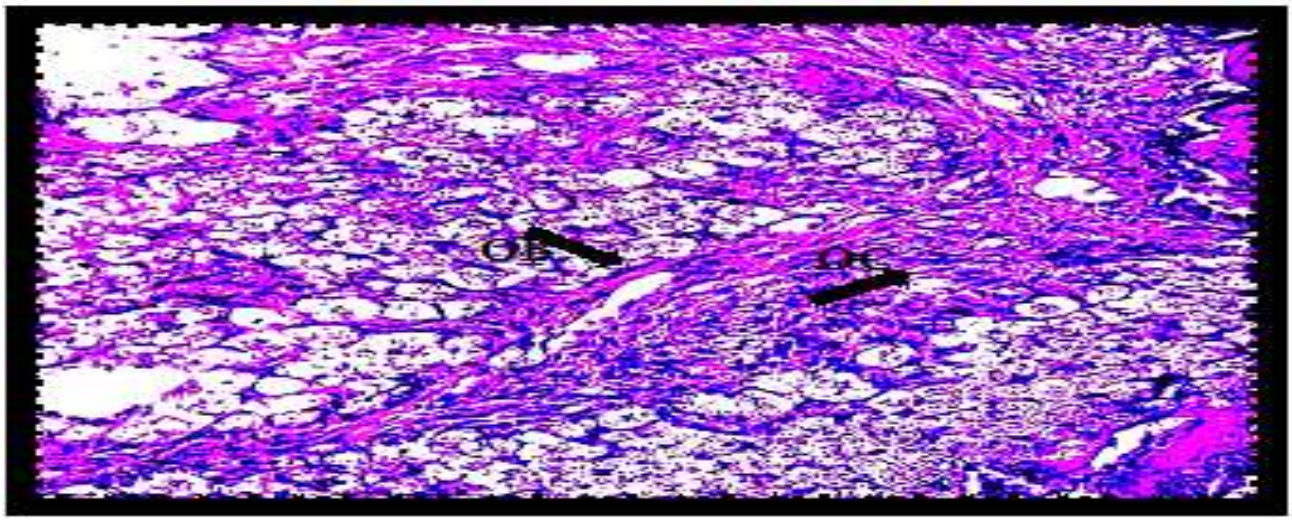

Figure 2: View of melatonin group of 1week duration shows bone spicules, osteoblasts (OB) ,and osteocytes (OC). H&EX10

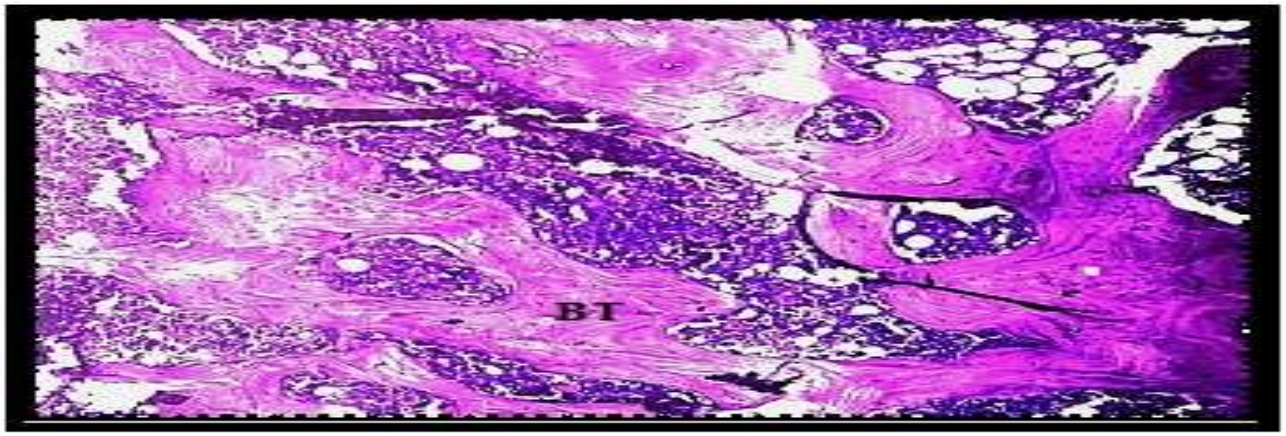

Figure 3: Microphotograph view of control group after2 weeks duration, shows deposition of new bone trabeculae (BT) . H&Ex10

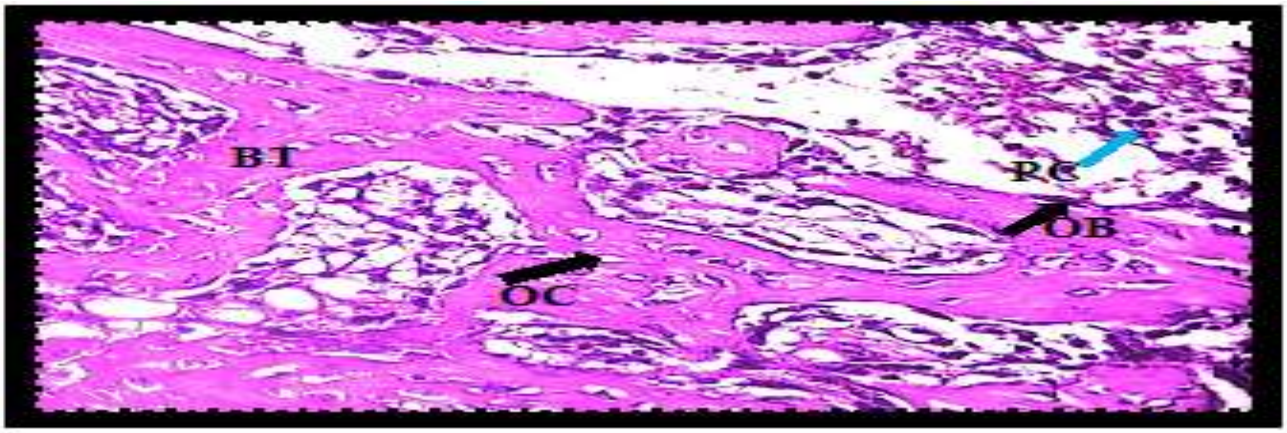

Figure4: View of melatonin group of 2 weeks duration shows progenitor cells (PC), bone trabeculae (BT),osteoblast (OB) and osteocytes (OC) . H&EX40

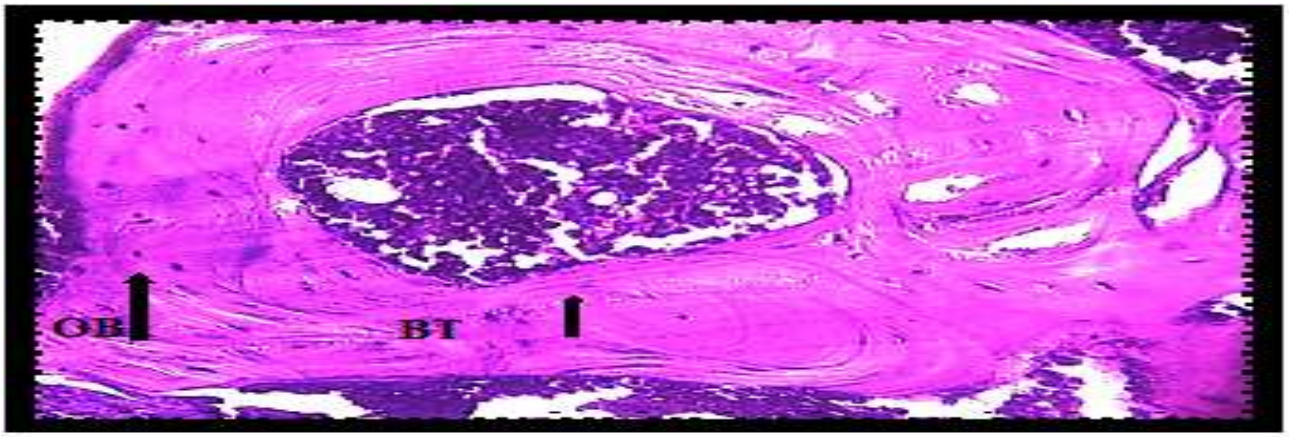

Figure5: View of defect site of control (C4) group after 4weeks duration shows bone trabeculae (BT) osteoblast cells (OB)and osteocytes arranged around haversian canal (arrow) .H&EX40

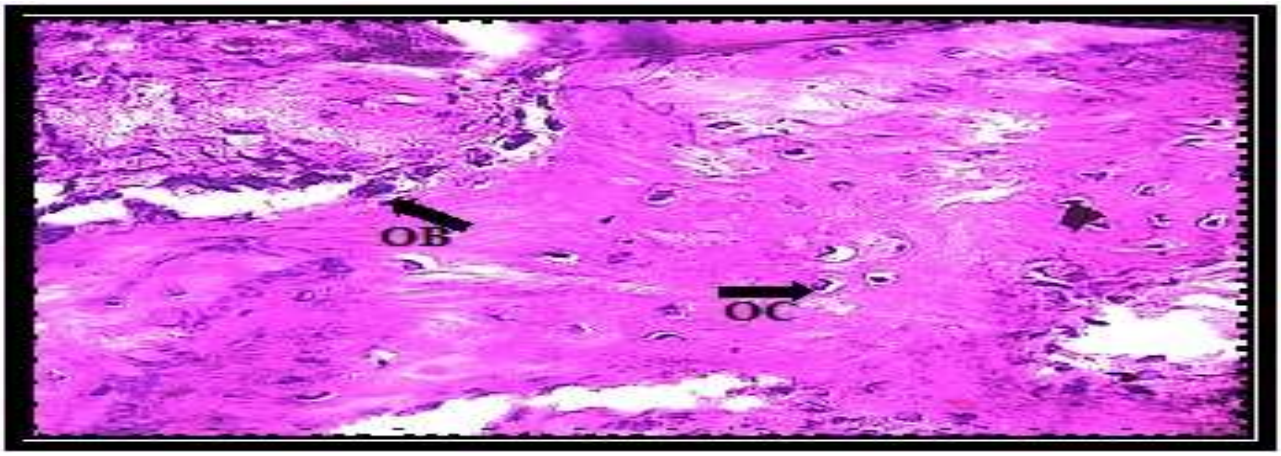

Figure 6: View of melatonin (M4) group after 4weeks duration shows osteoblast cells (OB) and osteocytes (OC). H&EX40

Histomorphometric Analysis Trabecula Area (TA), Trabecular Number (TN) and Bone Marrow Area (BMA)

Table 1 shows descriptive statistics of control and melatonin groups in 2 and 4 weeks durations. Mean values of (TA) and (TN) increased with time and were higher in melatonin group than in control group.

Whereas bone marrow area showed decrease in mean values with time and were higher in control group than in melatonin group.

Table 1: Descriptive statistics of control and experimental groups at different healing periods for TA, TN, BMA

Variables	Duration	Control group						Melatonin group					
		N	Mean	S.D.	S.E.	Min.	Max.	N	Mean	S.D.	S.E.	Min.	Max.
Trabecular Area	2weeks	8	0.112	0.007	0.002	0.106	0.119	8	0.129	0.005	0.002	0.124	0.133
	4weeks	8	0.120	0.005	0.001	0.116	0.124	8	0.137	0.003	0.001	0.134	0.141
Trabecular No.	2weeks	8	5.12	1.5	0.54	3.83	6.42	8	11.38	1.68	0.59	9.97	12.78
	4weeks	8	7.5	1.8	0.65	5.95	9.05	8	14.62	2.4	0.86	10.90	15.10
Bone marrow area	2weeks	8	0.142	0.011	0.003	0.132	0.0151	8	0.111	0.012	0.004	0.100	0.121
	4weeks	8	0.120	0.009	0.003	0.112	0.128	8	0.075	0.010	0.003	0.066	0.084

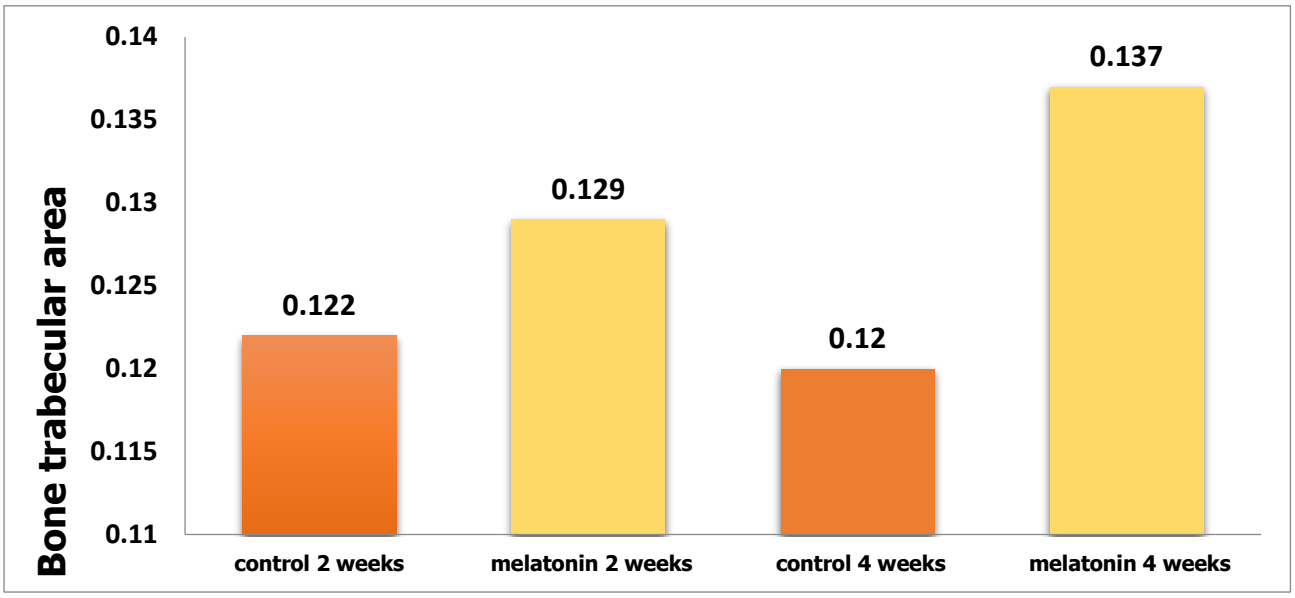

Figure 7: Comparison of mean values of trabecular area in studied group's indifferent durations

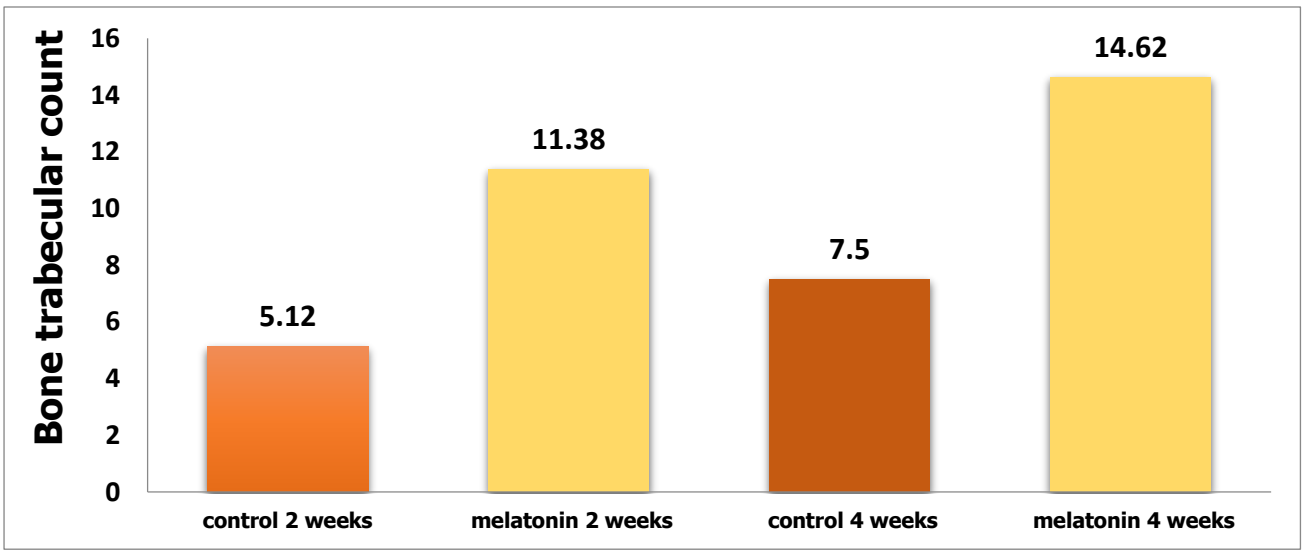

Figure 8: Comparison of mean values of trabecular number in studied groups in different durations

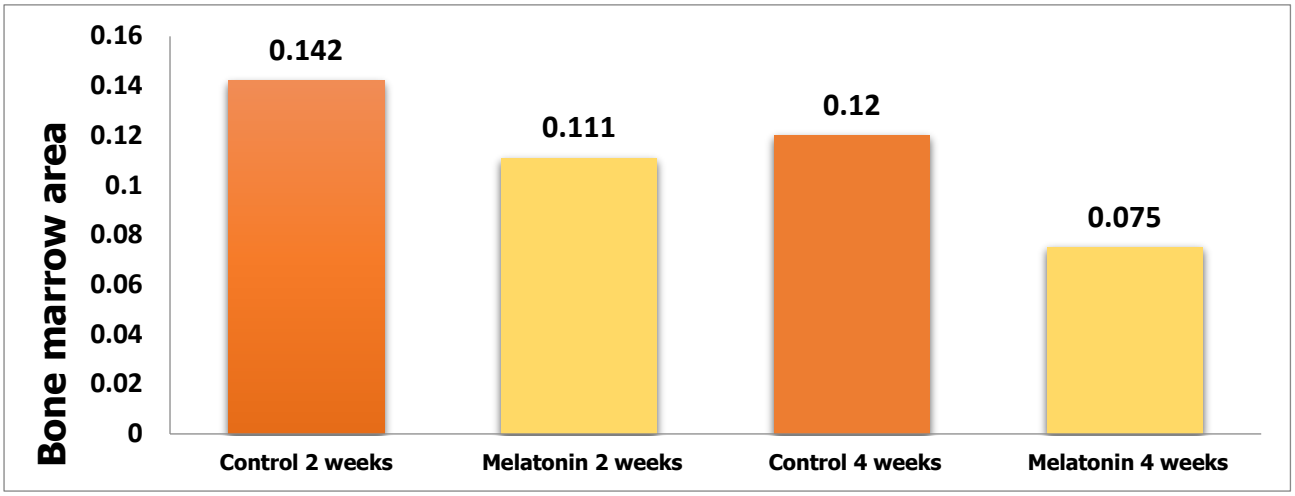

Figure 9: Comparison of mean values of bone marrow area in studied groups in different durations

Bone Cells

Table 2 shows mean values of osteoblasts and osteocytes which increased with time and were higher in melatonin group than in control group in different durations. Osteclasts recorded highest mean values in 2weeks duration of control group as shown in Figures (10, 11, 12).

Table 2: Descriptive statistics of control and experimental groups at different healing periods for bone cells

Variables	Duration	Control group						Melatonin group					
		N	Mean	S.D.	S.E.	Min.	Max.	N	Mean	S.D.	S.E.	Min.	Max.
Osteoblast	1 week	8	3.62	0.58	0.20	3.13	4.11	8	9.5	0.86	0.30	8.77	10.22
	2weeks	8	4.46	0.89	0.31	3.13	4.11	8	10.09	1.07	0.38	9.19	10.99
	4weeks	8	4.53	0.69	0.24	3.94	5.11	8	11.56	1.2	0.42	10.54	12.57
Osteoclasts	1 week	8	0.46	0.16	0.05	0.27	1.1	8	0.71	0.31	0.11	0.45	0.97
	2weeks	8	0.96	0.31	0.11	0.70	1.2	8	0.71	0.20	0.07	0.54	0.89
	4weeks	8	0.5	0.18	0.06	0.34	0.65	8	0.56	0.17	0.06	0.41	0.71
Osteocytes	1 week	8	3.56	0.25	0.09	3.34	3.77	8	9.53	1.25	0.44	8.48	10.58
	2weeks	8	3.71	0.38	0.13	3.39	4.04	8	9.96	1.09	0.38	9.05	10.88
	4weeks	8	4.56	0.85	0.30	3.84	5.27	8	11.18	1.1	0.39	10.26	12.11

N: number of rats, S.D.: standard deviation
S.E.M: standard error of mean, Min.: minimum, MAX. : Maximum

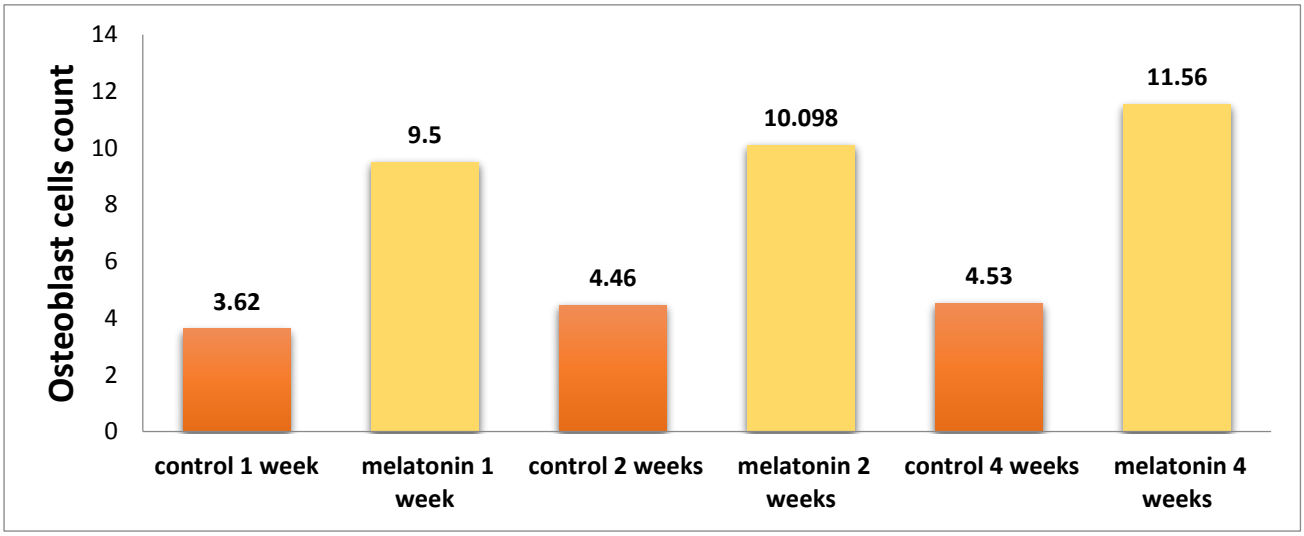

Figure 10: Comparison of mean values of osteoblasts in studied groups in different durations

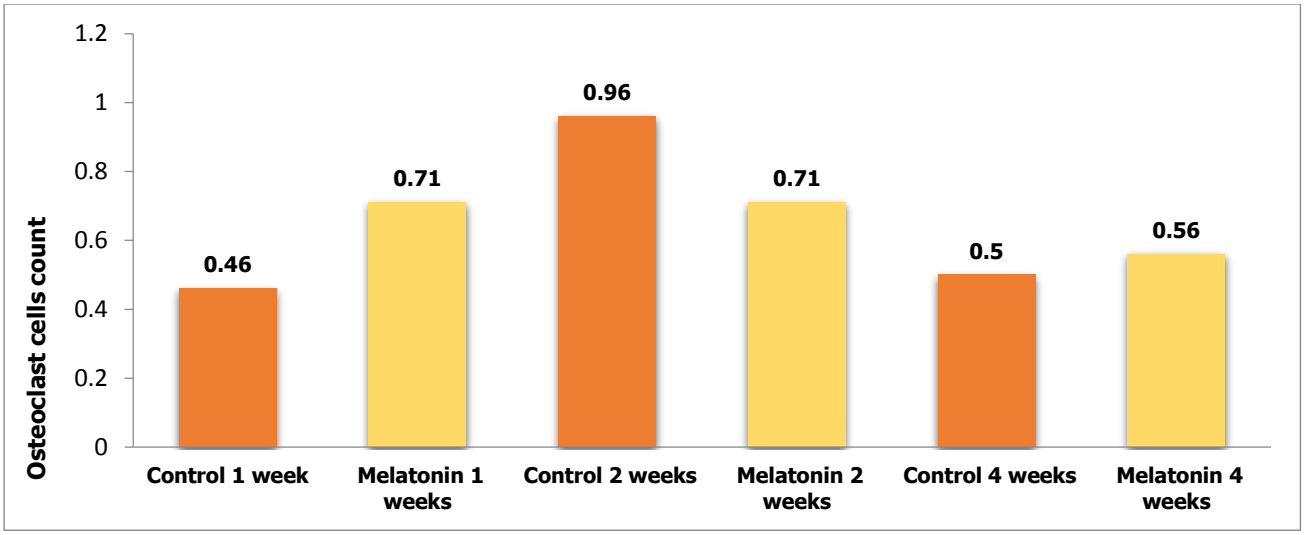

Figure 11: Comparison of mean values of osteoclasts in studied groups in different durations

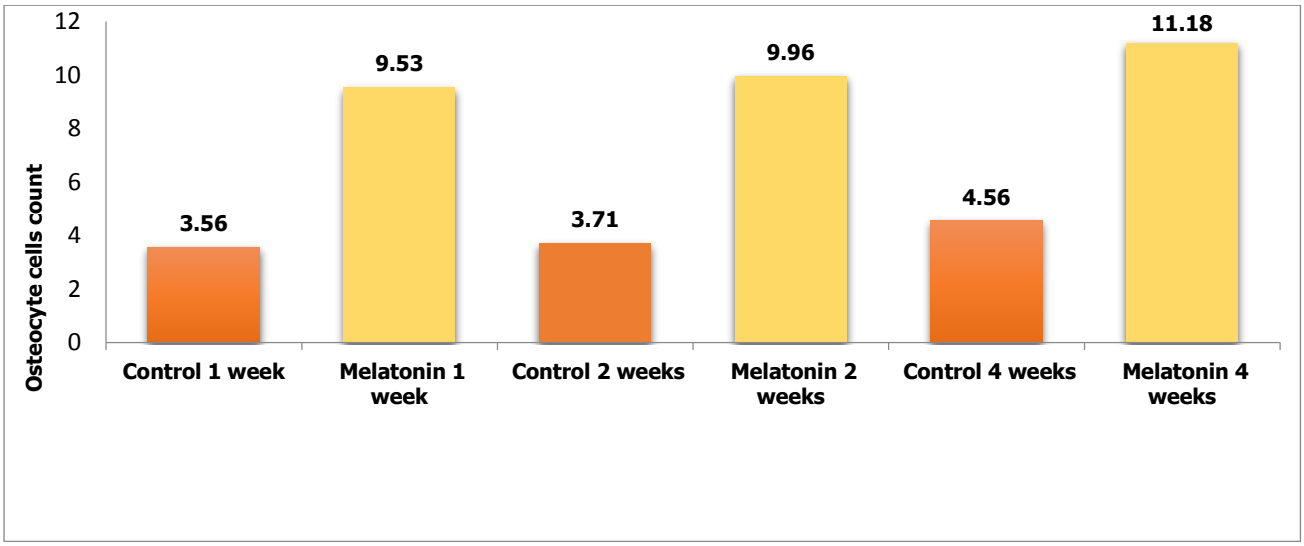

Figure 12: Comparison of mean values of osteocytes in studied groups in different duration

Discussion

The present study evaluated the effect of local exogenous melatonin application The histological examination of bone sections showed deposition of osteoid bone in all studied groups after one week and immature bone spicules rimmed by osteoblasts were observed more clearly in experimental (melatonin) group in agreement with findings of Calvo-Guirado and co- workers [8] who stated that significant osteoid matrix synthesis and mineralization accelerated was by early cell differentiation.

Microscopic examination of serial sections from the intervention site, performed 2 weeks after the start of the experiment showed early processes of bone healing in the area of the experimental defect, and thin bone trabeculae are present as reported by [9]in agreement with the findings of this study where bone trabeculae enclosing marrow tissue were illustrated by histological examination of bone sections of control group after 2 weeks .However this finding disagrees with other study [10] which stated that melatonin could increase the osteogenic effect by increasing the osteoblast cell proliferation and stimulating matrix activity, they investigated the effects of melatonin on healing of tibial bone defect model in rats where they noticed that the improvement did not occur in defects in the 14th day , while at 28 days, the beginning of trabecular bone formation was observed.

At 4 weeks more areas of bone formation were observed with thin and small bony trabeculae as reported by a previous study [11], while the present results showed dense mature bone filling defect sites in all studied groups after 4weeks. Descriptive statistical analysis of bone parameters (trabecular area and trabecular number) showed increased mean values with time and were higher in melatonin group while values of bone marrow

area decreased .Melatonin improved the bone trabecular microstructure of elderly rat including trabecular number (Tb. N) and trabecular thickness (Tb. Th), and decreased trabecular spacing (Tb. Sp) in elderly rats in agreement with present results regarding mean values of TA, TN, and BMA which correspond to marrow spacing[12].

A previous study showed that a small flattened and inactive osteoblasts were detected overlying the immature bone surfaces in the histological observations of calvarial defects at three-week period [13], this findings seem to disagree with our findings concerning number of osteoblasts as mean values increased during the transition from the 1,2 to 4weeks of healing intervals among all groups ,it could be explained by the direct action of applied materials on the differentiating and maturation of osteoblasts accelerating rate of matrix deposition and its corresponding calcification, where osteocytes were embedded which also showed increasing mean values with time .At the microscopic level, bone remodeling is produced in basic multi cellular units, where osteoclasts resorb a certain quantity of bone and osteoblasts form the osteoid matrix and mineralize it to fill the previously created cavity.

These units contain osteoclasts, macrophages, preosteoblasts and osteoblasts [14][8] in agreement with results obtained in present study where higher mean values of osteoclasts recorded in 2 weeks duration and decrease in four weeks duration as process of bone deposition and maturation is almost settled .

Conclusion

The study revealed that the local application of melatonin was effective in the enhancement of bone regeneration by acceleration and speeding up bone healing process.

References

1. Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA (2008) Dent J Molecular Mechanisms Controlling Bone Formation during Fracture Healing and Distraction Osteogenesis. J. Dent. Res., 87(2): 107-118.
2. Cardinali DP, Ladizesky MG, Boggio V, Cutrera RA, Mautalen C (2003) Melatonin effects on bone: Experimental facts and clinical perspectives. J. Pineal. Res., 34, 81–87.
3. Kotlarczyk MP, Lassila HC, O'Neil CK, D'Amico F, Enderby LT, Witt-Enderby PA, Balk JL (2012) Melatonin osteoporosis prevention study (MOPS): A randomized, double-blind, placebo-controlled study examining the effects of melatonin on bone health and quality of life in perimenopausal women. J. Pineal. Res. 52, 414-426.
4. Jie L, Hong-yu Z, Wen-Guo F, Wei-Guo D, Shen-Li F, Hong-Wen H, Fang H (2013) Melatonin influences proliferation and differentiation of rat dental papilla cells in vitro and dentine formation in vivo by altering mitochondrial activity. J. Pineal. Res. 54: 170-178.
5. Takeuchi H, Nagayama M, Imaizum Y, Tsukahara T, Nakazawa J, Kusaka Y, Ohtomo K (2009) Immunohistochemical analysis of osteoconductivity of ß –TCP (tricalcium phosphate) and carbonate apatite applied in femoral and parietal bone defects in rats. Dental materials Journal, 28(5):595-601.
6. Dave RH (2008) Overview of pharmaceutical excipients used in tablets and capsules. Drug Topics. Advanstar. 10/24/2008 http:// drugtopics.modernmedicine.com/drugtopics/ Top+News/ Overview-of- pharmaceutical-excipients- used-in-tabl/ArticleStandard/Article/detail/561047. Seen at 29/11/2017.
7. Vidal B, Pinto A, Galvão MJ, Santos AR, Rodrigues A, Cascão R, Abdulghani S,Caetano-Lopes J1, Ferreira A, Fonseca JE, Canhao H (2012) Bone histomorphometry revisited. Acta. Reumatol. Port., 37:294-300.
8. Calvo-Guirado JL, Gómez-Moreno G, Maté-Sánchez J E, López-Marí L, Delgado-Ruiz R, Romanos GE (2015) New bone formation in bone defects after melatonin and porcine bone grafts: experimental study in rabbits. Clin. Oral. Implants Res., 26(4):399-406.
9. Oana L, Miclăus V, Daniela Oros , Almăsan H , Ratiu C, Rus1 V, Pestean C, Ober C, Crecan C (2008) Annals of RSC. Experimental method of inducing a bone defect in rats and histological monitoring of the evolution of the healing process. , Lucia Bel., 111-114.
10. Koparal M, Irtegun S, Alan H, Deveci E, Gulsun B, Pektank G (2016) Effects of melatonin on tibia bone defects in rats. Int. J. Morphol., 34(2):763-769.
11. Amera A (2015) Histomorphometric and Biochemical Evaluations of Stem Cells for Osteogenesis and Neogenesis. Austin Journal of Dentistry. Austin J. Dent., 2(3): 1024.
12. Chu-Tong L, Wei-Fang L, Chin-Yi L, Haw-Ming H, Sheng-Wei Feng, Wei-Jen Chang (2015) A Novel Porcine Graft for Regeneration

of Bone Defects Eisner Salamanca Materials, (8):2523-2536).

13. Shyng YC, Devlin H, Sloan P (2001) The effect of streptozotocin induced experimental diabetes mellitus on calvarial defect healing and bone turnover in the rat. Int. J. Oral. Maxillofac. Surg., 30:70-4.)
14. Gil I, Alobera-Gracia MA, del-Canto-Pingarrón M, Fernández-Tresguerres-Hernández, Blanco-Jerez L (2006) Physiological bases of bone regeneration II. The remodeling process. Medicina. Oral, Patologia. Oral y. Cirugia. Bucal., 11(2):E151-7.

