

THE POTENTIAL EFFECT OF BISPHOSPHONATE ON TEETH ENAMEL AND DENTIN FORMATION IN BOTH NORMAL AND OSTEOPOROTIC ALBINO RATS

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ABSTRACT

Aim: The aim of the current study to establish the effect of Bisphosphonate on Teeth Enamel and Dentin Formation in Both Normal and Osteoporotic Albino Rats.

Materials and Methods: This study was included 4 groups of newborn rats, these groups were divided as follow: Group I (negative control), Group II (positive control), Group III (Bisphosphonate group), Group IV (Betamethasone & Bisphosphonate). The numbers of rats in each group were ten rats so the total numbers of rats were forty newborn rats with their average weight 5 grams then according to the date of euthanization of the rats at 10, 20 days; each group was divided into two subgroups. Injection of drugs in the dorsal back of rats were made. The specimens were processed for histological, histomorphometry and statistical analysis.

Results: Betamethasone (Group II) made over the developing molar teeth bone resorption but made increasing the thickness of enamel and dentin while Bisphosphonate (group III) made disorientation of odontoblastic layer and decreased the thickness of the mineralized dentin. Dental follicle of the 1st molar appeared discontinuous because it was invaded by bone spicules that reached dental organ at some points at the occlusal portion made deformity of enamel matrix and decreased the thickness of the enamel. In group IV: Both drugs made very thin layer of enamel but made little effect on dentin thickness

Conclusion: Betamethasone decreased dentin mineralization but increased its thickness and also decreased the mineralization of ename while Bisphosphonate decreased dentin mineralization and made distortion of odontoblastic layer inside pulp but made more increasing in its thickness.

KEY WORDS: Bisphosphonate, Betamethasone, Enamel and Dentin formation.

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INTRODUCTION

The crown of teeth is covered with enamel and it is an ectodermal in origin which differs from the other tissues of the teeth which are ectomesenchymal in origin. Regeneration of enamel is impossible. Mineral content of the Enamel is 96% Which contain calcium phosphate in the form of hydroxyapatite crystals and in the human body enamel represent the strongest and hardest tissue. The organic content of the enamel is 4% in the form of water and protein. ^(1,2)

The main bulk of the tooth is dentin, Enamel covers dentin of the crown and cementum covers dentin of the root. Inorganic content of the dentin is 70-75% in the form of hydroxyapatite crystals and Organic content of the dentin is 25-30% in the form of glycoproteins, collagen type 1 and protein. ^(3,4)

One of the important drugs that inhibit osteoclast function and prevent bone resorption are Bisphosphonate(Bps) so they were used in the treatment of osteoporosis, and also these drugs were used in the bone diseases treatment like idiopathic juvenile osteoporosis, fibrous dysplasia and hypercalcemia occurred in malignant diseases. ⁽⁵⁾

Corticosteroids have anti-inflammatory effect so these drugs inhibit irritation associated with inflammatory reaction and also promote the prostaglandins production. Prostaglandins promote vasodilatation and permeability of the blood vessels so inflammatory reaction decrease. Corticosteroids also have analgesic effect so these drugs were used in the treatment of pain associated with many different diseases. ^(6,7)

Corticosteroids promote osteoclast function which make bone resorption so the bone resorption exceed bone formation this lead to osteoporosis; which is a skeletal bone disorder. Osteoporosis decrease the quality and the quantity of the bone so the bone mass is more reliable to fracture. In this study, the effect of bisphosphonate on teeth enamel and dentin formation in both normal and osteoporotic albino rats will be investigated. ⁽⁸⁾

METHODS AND MATERIALS

Betamethasone in the form of a readymade ampoule of glucocorticoid was used to promote of the osteoporosis and also to be injected daily in the dorsal back of newly born albino rats with dose (100ng/g body wt. /day) was dissolved in water for twenty days.

Bisphosphonate in the form of the tablets was brought from the pharmacy and then was crushed it into powder form and then the powder was mixed with distilled water and syringe contained these mix with percentage 1:1 (powder: water) and then the rats were injected daily for twenty days with average dose 2.5 mg/kg/day.

Ten mother delivered forty newborn rats and their average weight 5 grams. Each mother delivered four new born rats. The rats were obtained from the animal house in Al Haram office in Giza. All rats were put into cages with room temperature at 23 and humidity 55. Mothers fed their newborn rats through breast milk. We maintained rat's cages under good ventilation during the experimental period.

The rules of experimental studies of the animal must be followed and accepted by Ethical Committee including their facilities diet and method of euthanization.

Rats were divided into four groups as follow: Group I (negative control), Group II (positive control), Group III (Bisphosphonate) and Group IV (Betamethasone & Bisphosphonate) then according to the date of euthanization; each group was divided into two subgroups at 10,20 days.

Observations of animals were made daily. For euthanization, the animals then were euthanized by anesthetic overdose of thiopental. After that dissection of the mandible were made. Collected specimens at days (10, 20) were obtained; sagittal sections were made in the molar area of the rats and then were processed for histological examination.

Histological Examination

The specimens were fixed for 24 hours in the neutral solution of the formalin. We used water to wash the specimens from formalin solution. Then we used EDTA to make decalcification of the specimen at PH 7.

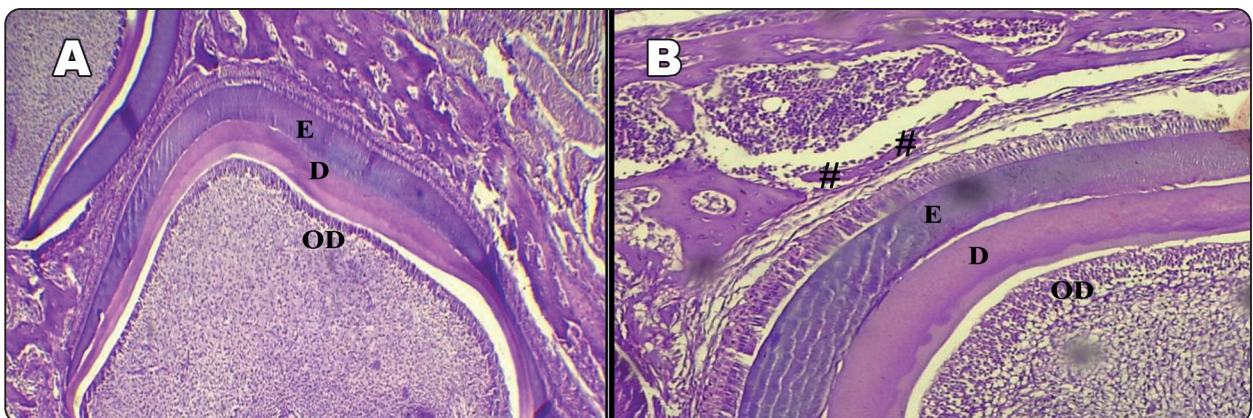
Then washing of the specimens by using water were made for removing decalcifying agent, then the dehydration of the specimens were made in ethyl alcohol and dry the specimens into 2 concentrates of xylene then the specimens were put into the paraffin wax. Then we used rotary microtome for sectioning of the specimens into thin sections (5 micron in thickness) then specimens were stained by (H&E) for histological examination under light microscope, histomorphometry and statistical analysis.⁽⁹⁾

RESULTS

At day 10, All rats specimens in the group II appeared with increasing in the thickness of enamel matrix and dentin and Odontoblastic layer appeared disoriented under cusp tips as compared with the control group (Fig. B) While All rats specimens in the group III appeared with decreasing in the thickness of enamel matrix and dentin and odontoblastic layer appeared also disoriented under cusp tips as compared with the control group. Dental follicle of the 1st molar appeared discontinuous

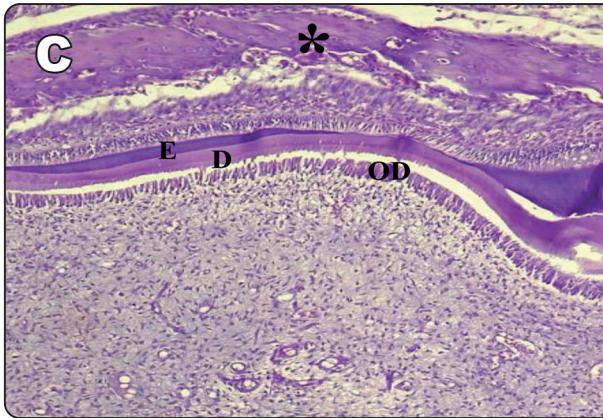
because it was invaded by bone spicules that reached dental organ at some points at the occlusal portion made deformity of enamel matrix (Fig. C). All rats specimens in group IV appeared with very thin layer of enamel matrix but made little effect on dentin thickness as compared with the control group and areas of increasing the number of bone spicules also were appeared (Fig. D)

At day 20, All rats specimens in the group II appeared with increasing the thickness of dentin, thin layer of enamel matrix and odontoblastic layer appeared more disoriented under cusp tips as compared with the control group (Fig. E) While All rats specimens in the group III appeared with multiple spots of enamel matrix, dentin appeared with more increasing in its thickness and odontoblastic layer appeared also more disoriented under cusp tips as compared with group I and II. Dental follicle of the 1st molar appeared discontinuous because it was invaded by bone spicules that reached dental organ at some points at the occlusal portion made deformity of enamel matrix (Fig. G). All rats specimens in group IV appeared with thin layer of enamel matrix and the largest thickness of dentin appeared in this group and areas of increasing the number of bone spicules also were observed (Fig. H). Dentin appeared with variation in the staining intensity (Fig. G&H).



Photomicrograph of control group at 10 day postoperative showing E (Enamel), D (Dentin) of normal thickness and OD (Odontoblastic layer) (H&E Orig. Mag. X100)

Photomicrograph of Betamethasone group at 10 day showing bone resorption over the tooth (#) and the thickness of enamel and dentin were increased (H&E Orig. Mag. X100)



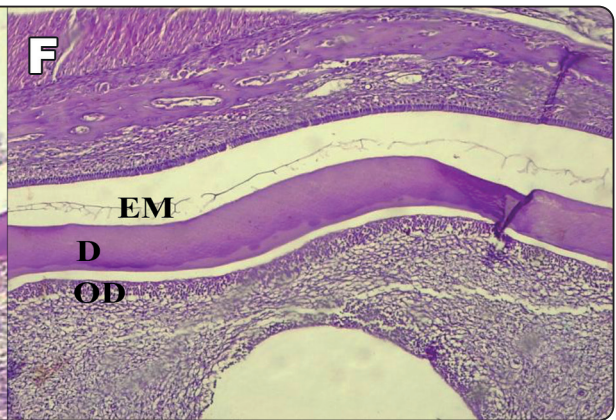
Photomicrograph of group III at 10 day showing increased of bone trabeculae (*) and the thickness of Enamel and dentin were decreased (H&E Orig. Mag. X100)



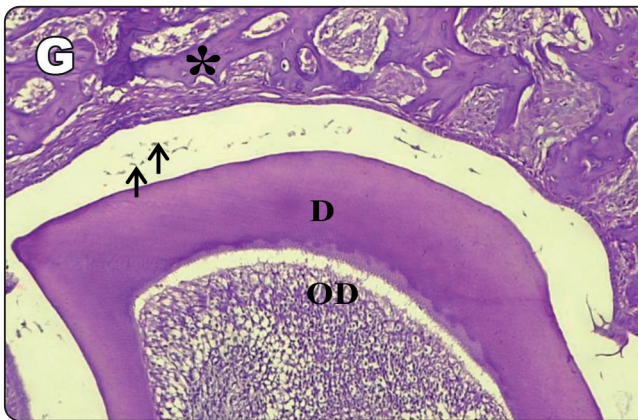
Photomicrograph of group IV at 10 day showing bone spicules were increased over the tooth (#), very thin layer of enamel and little effect on dentin thickness (H&E Orig. Mag. X100)



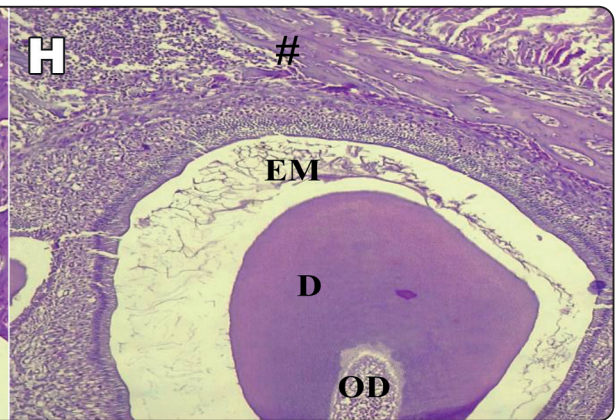
Photomicrograph of control group at 20 day showing ES (Enamel space), D (Dentin) of normal thickness and OD (Odontoblastic layer) (H&E Orig.Mag.X100)



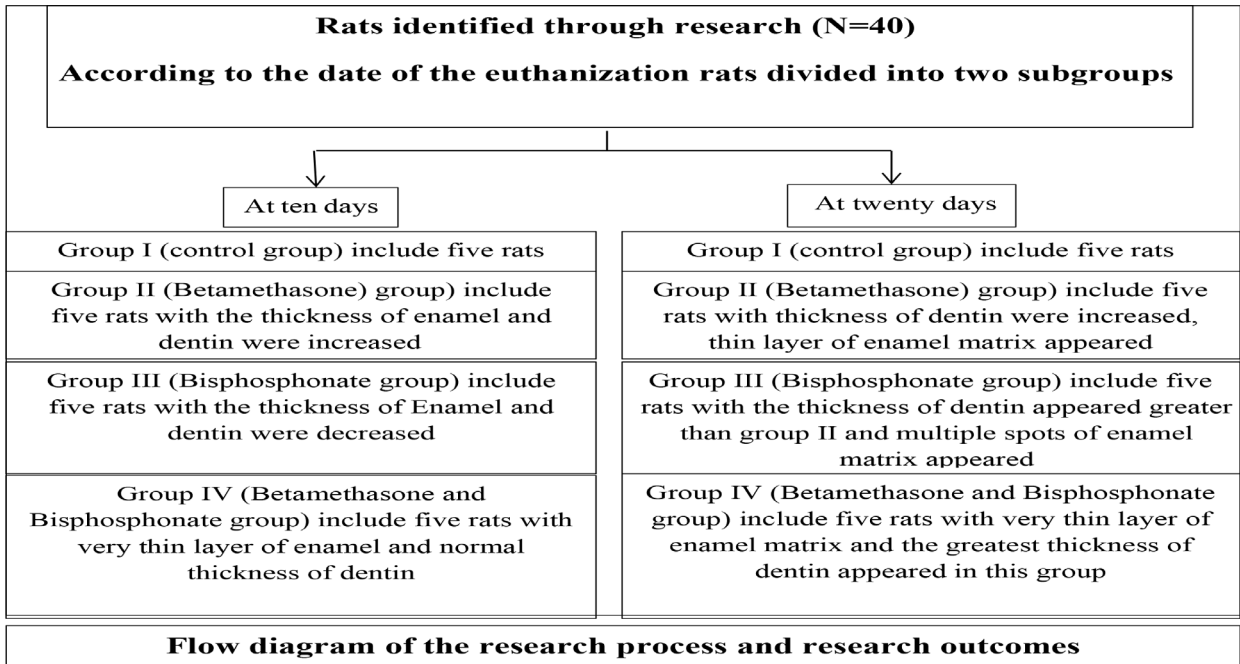
Photomicrograph of Betamethasone group at 20 day showing thickness of dentin were increased, thin layer of enamel matrix appeared (H&E Orig. Mag. X 100)



Photomicrograph of group III at 20 day showing increased the number of bone trabeculae (*), the thickness of dentin appeared greater than group II and multiple spots of enamel matrix appeared (arrows head) (H&E Orig. Mag. X100)



Photomicrograph of group IV at 20 day showing bone spicules were increased over the tooth (#), very thin layer of enamel matrix and the greatest thickness of dentin appeared in this group (H&E Orig. Mag. X100)



Comparison between groups at t 10 and 20 days and between different days at the same group for distance

Enamel

Comparison between intra groups at different time:

Statistical analysis showed significant difference between groups after 10 and 20 days at p value <0.01. Pair wise comparison showed significant difference between each group to another at 10 and 20 days except group 1 with group 2 after 10 days. After 10 days the high mean value was found in G2, followed by G1 and G4, while the lowest value was recorded in G3. On the other side After 20 days, the high mean value was found in G4, followed by G3 and G1, while the lowest value was recorded in G2 (Table 1, Fig 1).

Changing of distance according to time (Inter groups)

Statistical analysis showed significant difference between 10 and 20 days in same group at p value <0.01. According to G1 and G2 the distance was

decreased significantly after 20 days while in G3 and G4 the distance was significantly increased after 20 days compared with 10 days (Table 1, Fig 1).

Dentin

Comparison between intra groups at different time

Statistical analysis showed significant difference between groups after 10 and 20 days at p value <0.01. Pair wise comparison showed significant difference between each group to another at 10 and 20 days After 10 days the high mean value was found in G4, followed by G1 and G2, while the lowest value was recorded in G3. On the other side after 20 days, the high mean value was found in G4, followed by G3 and G1, while the lowest value was recorded in G2 (Table 1, Fig1).

Changing of distance according to time (Inter groups)

Statistical analysis showed significant difference between 10 and 20 days in same group at p value <0.01. According to G2 the distance was decreased significantly after 20 days while in G1, G3 and G4

the distance was significantly increased after 20 days compared with 10 days (Table 1, Fig 1).

Comparison between Enamel and dentin for distance after 10 and 20 days

Statistical analysis showed significant difference

between Enamel and Dentin for all groups after 10 and 20 days using independent sample T test at $P < 0.01$. The highest mean values of distance were recorded in Dentin for all groups than enamel after 10 and 20 days except in G2 after 10 days (table 2, Fig 2).

TABLE (1) Comparison between groups after 10 and 20 days and between different days at the same group for distance

	Enamel				Dentin			
	10 days	20 days	T test	P value	10 days	20 days	T test	P value
Group 1	181.5±14.7a	169.5±10.2c	8.2	<0.001**	269.1±11.6b	532.9±12.3c	18.3	<0.001**
Group 2	191.5±7.6a	54.2±4.5d	35.8	<0.001**	162.2±10.8c	100.2±8.9d	13.7	<0.001**
Group 3	11.8±3.5c	289.6±8.7b	89.1	<0.001**	33.6±3.6d	599.8±9.4b	160.5	<0.001**
Group 4	32.9±5.5b	912.7±7.8 a	135.2	<0.001**	645.3±12.8a	722.6±11.5a	8.3	<0.001**
F test	570.4	1228.2			2872.56	637.15		
P value	<0.001**	<0.001**			<0.001**	<0.001**		

** , means significant difference at $P < 0.01$

TABLE (2) Comparison between Enamel and dentin for distance at the same days

	10 days				20 days			
	Enamel	Dentin	T test	P value	Enamel	Dentin	T test	P value
Group 1	181.5±14.7	269.1±11.6	10.3	<0.001**	169.5±10.2	532.9±12.3	24.3	<0.001**
Group 2	191.5±7.6	162.2±10.8	4.9	<0.001**	54.2±4.5	100.2±8.9	11.6	<0.001**
Group 3	11.8±3.5	33.6±3.6	9.2	<0.001**	289.6±8.7	599.8±9.4	17.2	<0.001**
Group 4	32.9±5.5	645.3±12.8	106.2	<0.001**	912.7±7.8	722.6±11.5	13.7	<0.001**

** , means significant difference at $P < 0.01$

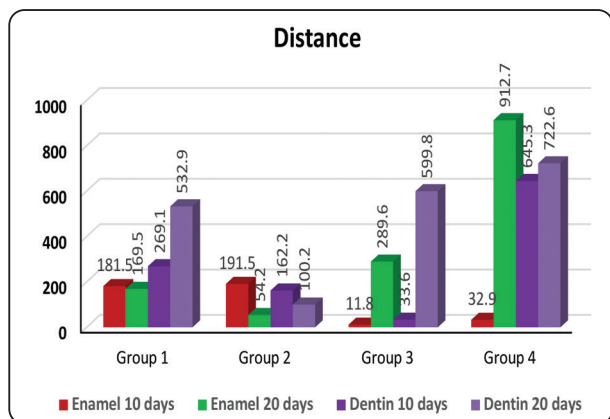


Fig. (1) Comparison between groups after 10 and 20 days in enamel and dentin for distance

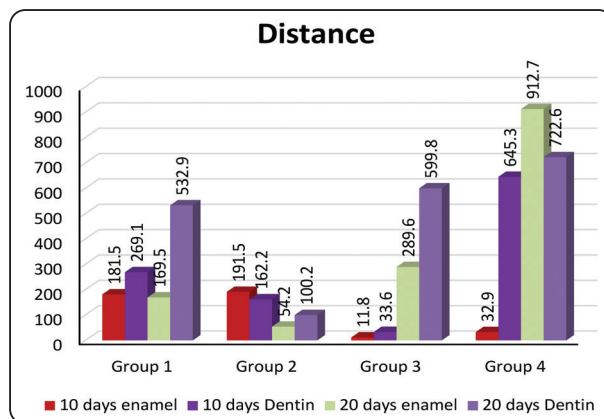


Fig. (2) Comparison between Enamel and dentin for distance after 10 and 20 days for distance

Comparison between groups at t 10 and 20 days and between different days at the same group for width

Enamel

Comparison between intra group at different time

Statistical analysis showed significant difference between groups after 10 and 20 days at p value <0.01. Pair wise comparison showed significant difference between each group to another at 10 and 20 days except group 2 with group 3 after 10 days. After 10 days the high mean value was found in G1, followed by G4, while the lowest value was recorded in G2 and G3. On the other side After 20 days, the high mean value was found in G4, followed by G3 and G1, while the lowest value was recorded in G2 (Table 3, Fig 3).

Changing of distance according to time (Inter groups)

Statistical analysis showed significant difference between 10 and 20 days in same group at p value <0.01. According to G2 the width was decreased significantly after 20 days while in G1, G3 and G4 the distance was significantly increased after 20 days compared with 10 days (Table 3, Fig 3).

Dentin

Comparison between intra groups at different time

Statistical analysis showed significant difference between groups after 10 and 20 days at p value <0.01.

Pair wise comparison showed significant difference between each group to another at 10 and 20 days After 10 days the high mean value was found in G4, followed by G1 and G2, while the lowest value was recorded in G3. On the other side after 20 days, the high mean value was found in G3, followed by G4 while the lowest value was recorded in G2 and G1 (Table 3, Fig 3).

Changing of distance according to time (Inter groups)

Statistical analysis showed significant difference between 10 and 20 days in same group at p value <0.01. According to G1, G2 and G4 the width was decreased significantly after 20 days while G3 only the distance was significantly increased after 20 days compared with 10 days.

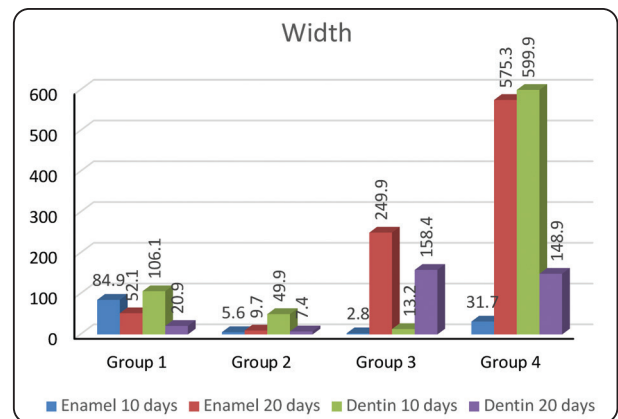


Fig. (3) Comparison between groups after 10 and 20 days in enamel and dentin for width

TABLE (3) Comparison between groups at t 10 and 20 days and between different days at the same group for width

	Enamel				Dentin			
	10 days	20 days	T test	P value	10 days	20 days	T test	P value
Group 1	84.9±10.6a	52.1±12.6c	9.3	<0.001**	106.1±18.1b	20.9±4.93c	78.4	<0.001**
Group 2	5.6±2.6c	9.7±3.2d	4.1	<0.001**	49.9±9.8c	7.4±2.52d	18.9	<0.001**
Group 3	2.8±0.9c	249.9±15.0b	67.5	<0.001**	13.2±6.6d	158.4±7.4a	57.4	<0.001**
Group 4	31.7±5.6b	575.3±13.1a	94.3	<0.001**	599.9±6.2a	148.9±6.9b	45.3	<0.001**
F test	192.13	782.71		<0.001**	2785.21	352.57		
P value	<0.001**	<0.001**			<0.001**	<0.001**		

** , means significant difference at P<0.01

TABLE (4) Comparison between Enamel and dentin for width at the same days

	10 days				20 days			
	Enamel	Dentin	T test	P value	Enamel	Dentin	T test	P value
Group 1	84.9±10.6	106.1±18.1	17.3	<0.001**	52.1±12.6	20.9±4.93	8.6	<0.001**
Group 2	5.6±2.6	49.9±9.8	14.9	<0.001**	9.7±3.2	7.4±2.52	4.8	0.041*
Group 3	2.8±0.9	13.2±6.6	7.2	<0.001**	249.9±15.0	158.4±7.4	9.3	<0.001**
Group 4	31.7±5.6	599.9±6.2	125.8	<0.001**	575.3±36.1	148.9±6.9	24.9	<0.001**

** , means significant difference at P<0.01

Comparison between Enamel and dentin for width after 10 and 20 days

Statistical analysis showed significant difference between Enamel and Dentin for all groups after 10 and 20 days using independent sample T test at P<0.01. The highest mean values of width were recorded in Dentin for all groups than enamel after 10 and 20 days except in G1 and G2 after 20 days (Table 4, Fig 4).

Statistical analysis:

All data calculated, tabulated, and statistically analyzed using suitable statistical tests as follows. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). Paired sample T test was used to compare between different time (10 and 20 day). Also, independent samples were used to compare enamel and dentin at the same time. One way ANOVA (Analysis of variance) was used to compare between the groups under study. Bonferroni post hoc test was performed for pair wise comparisons among the groups. P value ≤ 0.05 is considered statistically significant. Statistical analysis was performed using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels<0.05 (P- Value)

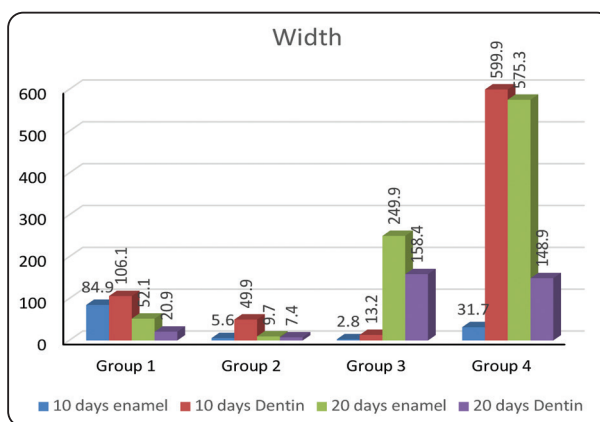


Fig. (4): Comparison between Enamel and dentin for width after 10 and 20 days for width

DISCUSSION

Introduction of osteoporosis was occurred by Betamethasone. Injection of Bisphosphonate in the newborn albino rats were made to counteract osteoporosis and the observation of the action of Betamethasone and Bisphosphonate on tooth enamel and dentin were done. These drugs are soluble in water so their injection in the rats are easy and these drugs are available in many places, administration of this drugs are easy, economic, absence of side effects and the powder preparations of these drugs are effective. (10)

The teeth of Albino rats have the same developmental stages as humans so we used them in

this study to facilitate the study of Bisphosphonate effects on tooth enamel and dentin during development of the teeth. ⁽¹¹⁾

Bisphosphonate are used in the treatment of many pediatric bone diseases. Administration of Bisphosphonate to children made alterations in enamel and dentin of the teeth in which odontogenesis are taking place and also Bisphosphonate are used in the treatment of adult bone disorders like postmenopausal osteoporosis and cancer chemotherapy. These drugs are used in malignant tumor metastasis prevention and prevention of the pain of the bone caused by radiotherapy. ⁽¹²⁾

This research showed that the injection of Bisphosphonate to rats dorsal back from day one to 20 days decrease the mineralization of dentin and also decrease the thickness of enamel matrix. This might be explained that Bisphosphonate affected enamel and dentin formation during the development of the teeth. ⁽¹³⁾

The bone crypt resorption over the erupting teeth didn't occur. Enamel organ was invaded by bone trabeculae this lead to make enamel deformity, since enamel organ cells are responsible for formation of enamel and also one can speculate that invasion of this cells by bone trabeculae affect the thickness of the enamel which normally covers dentin. ⁽¹⁴⁾

Enamel and dentin hypomineralization caused by Bisphosphonate which have inhibitory effects on mineralization of enamel and dentin. In contrast, these findings have not been reported in the group treated with Betamethasone. ⁽¹⁵⁾

CONCLUSIONS

Betamethasone decreased dentin mineralization but increased its thickness and also decreased the mineralization of enamel. Bisphosphonate decreased dentin mineralization and made distortion of odontoblastic layer inside pulp but made more increasing in its thickness.

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