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Natural products extract effect on bone integration around orthodontic micro-implant: An experimental study

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Abstract

Background and objective: Bone integration around orthodontic implant is a matter of concern for their stability. This study was conducted to estimate calcium and phosphorous level in after insertion of orthodontic implant.

Methods: Twenty five white mature male rabbits, classified into 5 groups: one control and four experimental, with five rabbits for each group were used. Fifty orthodontic implants were used, 2 micro-implant for each tibia. Four different natural products extract were used in this study that included Curcumin 15mg/kg, Nigella Sativa oil 0.25 ml/kg, Cissus Quadrangularis 500mg/kg and Virgin Coconut oil 1 ml/kg. Each product was given to certain experimental group started from the day of implant insertion for four weeks healing period. The biochemistry evaluation was conducted involving calcium and phosphorus level in serum.

Results: Significant difference in serum calcium levels were detected between Curcumin, Nigella Sativa oil from side and control group on the other side.

Conclusion: Systemic Curcumin and Nigella sativa oil may be used for possibly enhancing bone response around orthodontic implant as reflected by lower serum calcium level as compared to control group.

Keywords: Calcium and phosphorus, Natural products, Orthodontic implant.

Introduction

Many efforts have been spent by the researchers and clinicians to use the implant as anchorage unit in orthodontics.[1] Linkow[2] in his case report that is considered as the first published work in this field, used mandibular blade-vent implant with class II elastic to retract maxillary incisors. After that, in the last few years, anchorage reinforcement becomes possible and wide spread with mini-implants which proved to provide reliable anchorage.[3,4] For orthodontic implants to be successful, several factors should be taken in consideration such as bone quality and bone quantity.[5,6] The bone and its surrounding structures is considered as one of the most important factors that may affect the implant success rate. Bone healing is a local process that has an effect on systemic mineral homeostasis.[7] The major component and essential ingredient of human bones is a mineral form of calcium and phosphorus called hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The calcium and phosphorus components of these crystals are derived from the blood, where the calcium level in blood would be lowered during bone formation but phosphorus elevated this reflect that their percentages are inversely proportioned.[8] For that reason, sufficient calcium and phosphorus is necessary to ensure the proper balance of essential minerals in order to promote re-mineralization of bones.[9] A number of natural products were used since before for controlling bone metabolism in an attempt to promote anabolic effect or limiting/ suppressing the catabolism. Also, Vitamin E[10] added to natural herbal products such as Eurycomalongifola, Labisiapumila that mainly extracts from the whole plant or specific parts of the

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plants, such as the fruit, leaves, or roots.[11] According to our best knowledge, limited information is available regarding the effects of the natural products on calcium and phosphorous level after insertion of orthodontic or even surgical implants. Thus, the purpose of this study was to estimate the effect of Curcumin, Nigella Sativa, Cissus Quadrangularis and Virgin Coconut oil extracts on calcium and phosphorous level using biochemical evaluation methods.

Methods

In this study 50 sterile orthodontic micro implants were used with diameter of 1.3mm and length of 5mm (Dentos, AbsoAnchor, SH1312-5/ tapered type, Dentos Inc. 1-5, Galsan-Dong, Dalseo-Gu, Daegu, Korea 704-900). Twenty five healthy mature male rabbits weight 2-2.25 kilogram, 7-10 months-old, were used after receiving the bioethical approval of animal care from the University of Mosul in 24/11/2013. These rabbits were divided into 5 groups, each group 5 rabbits: First group was control that subjected to implant insertion but not given natural products. The remaining four groups were experimental that were subjected to implantation and given natural products. The implantation procedures were common to all animals and consisted of placement of two implants 10mm between each other in medial surface of the right tibia of each animal. All operations were performed under sterile conditions in a certain operating room, prepared for this purpose. Immediately before the surgery, the animals were anesthetized with intramuscular injection of 0.2 ml/kg body weight Ketamine 10% (KEPRO B.V. - Maagdenburgstraat 38-7421 ZE Deventer-Holland) and 0.15 ml/kg b.w. Xylazine 2% (alfasan.Woerden-Holland).[12] The surgical area anaesthetized with 1ml local anesthetic solution 2% Xylocain. The hair on the medial surface of the right leg clipped and the skin wiped thoroughly with Betadine solution (10% povidine iodine topical solution, purdue products L.P., Stamford) with sterile surgical gauze. An incision of approximately 20 mm in length down parallel to longitudinal axis of the tibia, in the medial aspect was conducted. The skin, fascia and muscles were dissected then the periosteum was stripped and elevated denuding the bone in medial aspect of tibia (Figure 1). Two implantation holes about 10 mm apart were drilled with a 1 mm rounded drill under profuse sterile saline-solution irrigation and at low rotary speed (Figure 2).

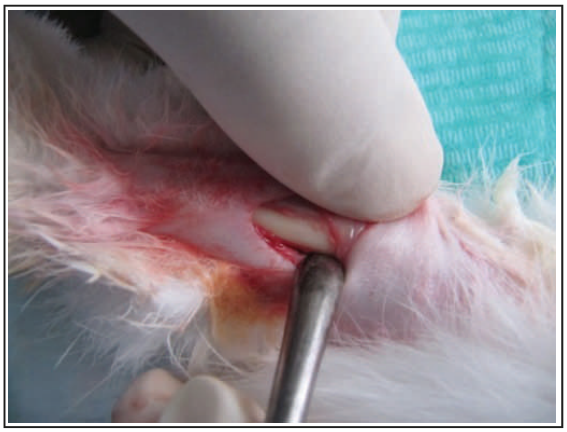

Figure 1: Soft tissue incision and bone exposure.

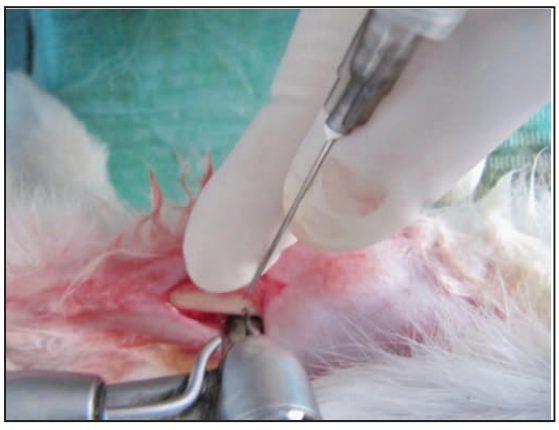

Figure 2: Implant hole preparation in tibia.

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The implants were threaded in the tibia with a manual driver with the full length of the serrated part inside the bone, till reaching the neck of the implant head collar (Figure 3, 4) keeping their longitudinal axes parallel to each other and perpendicular to the external cortical tibia. The periostium and deep fascia were repositioned in their original place and the skin was sutured (Figure 5) with black silk suture (3-0). The rabbit was then placed in postoperative recovery. Once fully recovered, the rabbits were placed in their respective holding areas. Then the natural products loaded for experimental groups as follows:

1. Curcumin: For first group one capsule of curcumin "CurcuVET-SA150/ Curcumin complexes with phosphatidylcho-line for superior bioavailability/ pure ingredient" (Throne Research, Inc. p.o. Box 25 Dover, ID 83825 USA) which contain 10mg/ kilogram of curcumin was given for each rabbit by dissolution of capsule content in distilled water and given directly through oral gavages". The dose was selected for this study taken with regard to different studies worked on bone metabolism using curcumin which range from 10-10000 mg/ Kg BW.[13-15]

2. Nigella Sativa oil (NSO): For this group, "Pure cold pressed 100ml Black Seed oil from Nigella Sativa seeds 0.25 ml/ Kilogram (kg) body weight (b.w.)" (The Blessed Seed oil/ Black seed oil specialist/ Beverley, United Kingdom), given freshly through oral gavages. Nigella Sativa oil dose selection was based on safety dose level.[16,17]

3. Cissus Quadrangularis (CQ): For this group, 500 mg/ kilogram b.w. "100% pure organic Cissus Quadrangularis extract by Keter Wellness/ under strict of good manufacturing practice (GMP) guidelines/ United State of America" (capsule weight 1gm) by dissolution of capsule content in distilled water. The dose was selected in reference to other studies which range from 500-750mg/kg.[18,19]

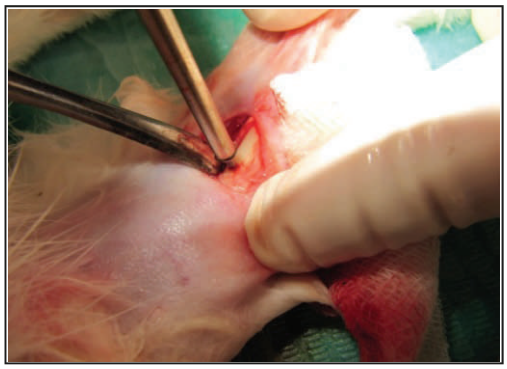

Figure 3: Screw insertion in tibia.

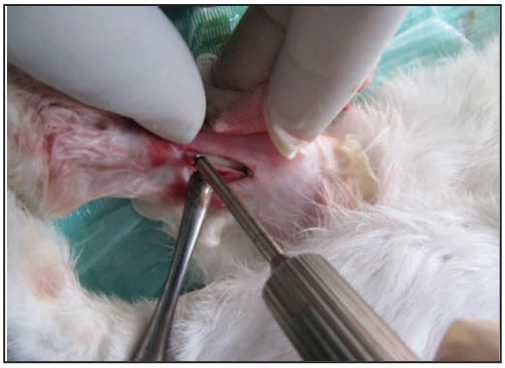

Figure 4: Two implants inserted with 10mm distance in between.

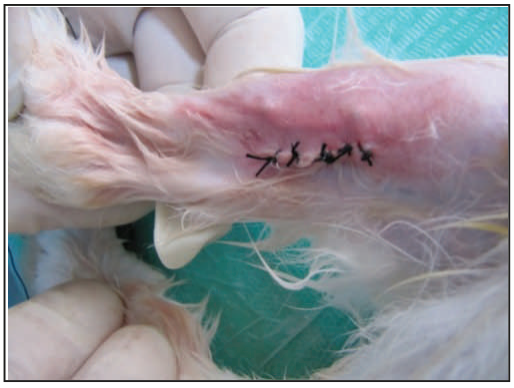

Figure 5: Skin suturing.

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4. Virgin Coconut oil (VCO): For this group, 1 ml/ kg b.w. Organic Pure Raw (Live superfoods, 20739, High Desert Court Bend, Oregon 97701). The dose selection was based on previous studies which range from 1-2 ml/ kg.[20,21]

All of these natural products were given as a loading dose for 4 weeks, started from the day of implant insertion. After 4 weeks healing period all of the rabbits were sacrificed in the same specially prepared theater under same circumstances. The rabbits were fasted overnight till morning for 12 hours. The sacrificing process was conducted by slaughtering with sterile large surgical blade to collect the blood sample for serum isolation. Once slaughtering started few blood drops were left down, not used to avoid hemolysis of blood sample, then the remaining gusher blood was collected in sterile glass test. After complete collection of blood in the serum separator tubes, serum collection achieved by leaving the blood inside the separating tubes for 30 minutes to allow for clotting to be achieved at room temperature. After centrifuging the blood inside the test tubes with centrifuge (EBA-20/ Hettich-ZENTRIFUGEN/ China) at a rate of 3000 turn/ 10 minutes. The serum drawn from the test tube with micro-pipette the extracted serum then putted in Eppendorf tubes which held in Eppendorf rack and putted in refrigerator at -20 co for serum preservation till the biochemistry test which did after two weeks. Prior to assay, the frozen sample brought to room temperature slowly and mixed gently. The biochemical evaluation involve measuring serum calcium, which was determined in an ordinary method using atomic absorption spectrophotometer (Spectrophotometer-Germany) milligram/ deciliter (mg/dl) level in serum. Serum phosphorus was determined using auto analyzer (Cobas Integra 400 plus/ Rouch-Germany) mg/dl level in serum. The method of analysis is shown in Figure 6.

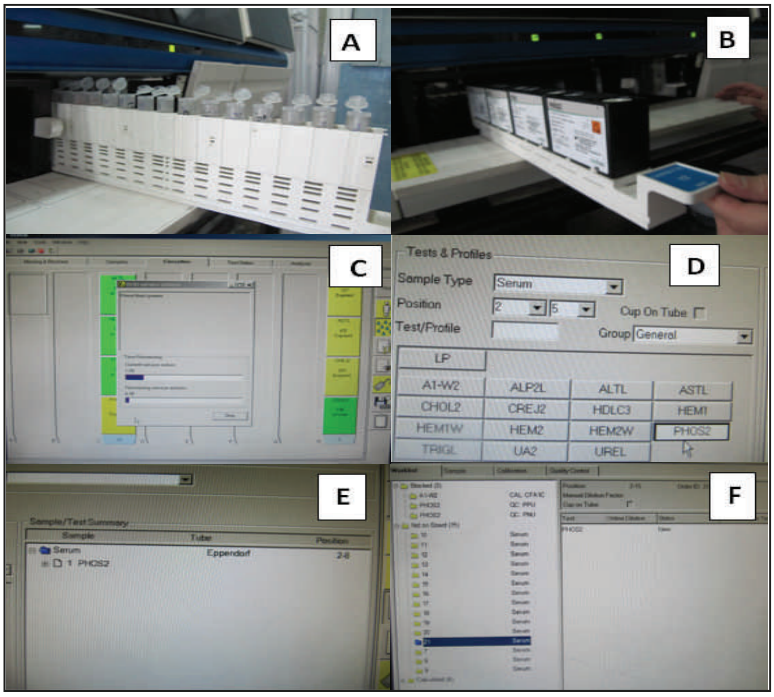

Figure 6: Auto analyzer for serum biomarker analysis: (A) Eppendrofs with serum loaded in the machine. (B) Loading the container specific for the biomarker to be measured. (C) Switch on the analyzer software. (D) Selection of the marker for analysis. (E) Selecting serum specimen in sample/ test summary. (F) Click the icon of each marked specimen to know their serum concentration.

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Results

The descriptive statistics for the study groups are presented in Table 1. A one-way analysis of variance (ANOVA) was conducted to compare the effect of the study materials on calcium and phosphorus level in the blood. There was a significant effect of these products on calcium level in the blood and there were no statistically significant differences between group means for phosphorus groups (Table 2).

Table 1: The descriptive statistics including means and standard deviation of serum calcium and phosphorus for the study groups.

Study groups	Mean	Std. Deviation
Control calcium	14.09	1.07
Curcumin calcium	12.06	0.66
Cissus Quadrangularis calcium	12.84	0.69
Nigella Sativa calcium	11.86	0.38
Vigin Coconut oil calcium	13.28	0.48
Control phosphorus	4.63	0.85
Curcumin phosphorus	6.18	1.62
Cissus Quadrangularis phosphorus	5.07	1.09
Nigella Sativa phosphorus	4.83	1.05
Virgin Coconut phosphorus	5.20	1.37

Table 2: Represents the one way analysis of variance of calcium and phosphorus for the study groups.

		Sum of Squares	df	Mean Square	F	*P* value
Calcium groups	Between Groups	16.721	4	4.180	8.543	0.001
	Within Groups	9.786	20	.489		
	Total	26.507	24			
Phosphorous groups	Between Groups	7.168	4	1.792	1.184	0.348
	Within Groups	30.263	20	1.513		
	Total	37.431	24			

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Scheffe's test was used to make post hoc comparisons among calcium groups (Table 3). This test showed that the mean value of Curcumin and Nigella Sativa oil differed significantly at P <0.05. However, the mean value of control group was not significantly different from the other groups.

Table 3: Scheffe's multiple comparisons for the calcium analysis.

Variables		Mean difference	SE.	*P* value
Control vs.	Curcumin	2.03	0.44	0.004
	Cissus quadrangularis	1.25	0.44	0.130
	Nigella sativa	2.23	0.44	0.002
	Virgin coconut oil	0.81	0.44	0.508
Curcumin vs.	Control	2.03	0.44	0.004
	Cissus quadrangularis	0.78	0.44	0.553
	Nigella sativa	0.20	0.44	0.995
	Virgin coconut oil	1.22	0.44	0.150
Cissus quadrangularis vs.	Control	1.25	0.44	0.130
	Curcumin	0.78	0.44	0.553
	Nigella sativa	0.98	0.44	0.331
	Virgin coconut oil	0.44	0.44	0.908
Nigella sativa vs.	Control	2.23	0.44	0.002
	Curcumin	0.20	0.44	0.995
	Cissus quadrangularis	0.98	0.44	0.331
	Virgin coconut oil	1.42	0.44	0.069
Virgin coconut oil vs.	Control	0.81	0.44	0.508
	Curcumin	1.22	0.44	0.150
	Cissus quadrangularis	0.44	0.44	0.908
	Nigella sativa	1.42	0.44	0.069

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Discussion

Bone integration or optimizing bone formation around orthodontic implant is a matter of concern. Identification of a substance with a positive effect on bone integration would possibly improve implant stability and could have a significant clinical relevance on implant failure. Recently, many investigations have been carried out on factors which may be able to increase the speed and quantity of bone formation around dental implants.[22-25] This study tried to use four types of natural products to test their possible effect on bone integration around orthodontic implant represented by serum biochemistry evaluation. The study condition is differing from previous studies, as there was traumatic injury, inflammation and bone response pathologic remodeling.[26,27] Significant differences for serum calcium on comparing Curcumin and NSO groups with control one were detected with lower serum calcium level for experimental groups. VCO and CQ groups also showed low serum calcium level in comparison with control group although statistically not significant referring to mean. These results cab probably be explained by the natural products activity at osseous level as mentioned above. These may be the result of increased calcium intake by the bone during formation and remodeling process. This result could come in agreement with the result achieved by Komnenou et al,[28] who conducted a study on 83 dogs, with closed long bone diaphyseal fractures treated surgically. The serum calcium and phosphate were evaluated at day of admission, 10, 20, 30 then 2 months then at 5 months. Their study results showed that Serum Phosphorus and Calcium changes followed a proportional and inverse pattern. This inverse relation between serum calcium and serum phosphorus was further documented by Kini and Nandeesh,[29] which be under the control of parathyroid hormone that regulates serum calcium and phosphorus concentrations through its receptor-mediated, combined actions on bone. In that on continues release of parathyroid hormone lead to bone resorption and opposite to it, bone formation occur with intermittent release of parathyroid hormone. Animal studies have demonstrated the importance of phosphorus, in conjunction with calcium, for bone development.[30] Their results showed that calcium and phosphorus are co-dependent, and that both minerals are critical to support soft tissue and bone growth.[29] In a study by Cohen et al on bone graft, stated that no evidence can be withdrawn from data for local preferential transfer of calcium to callus from grafts or bone store adjacent to the grafted area.[31] On contrary, all of the data may be used to support the hypothesis that calcium in callus or newly formed bone is completely from serum. So result in lower blood serum calcium level. At less than two weeks the blood contained no activity, and at more than three weeks of bone healing the specific activity of blood had dropped below the level at which the most active bone formation could possibly have been formed. The present study result come in accordance with Cohen et al[31] study and also agreed with Deka et al in 1994 which showed a lower serum calcium level accompanying bone fracture healing using Cissus Quadrangularis.[32] The present study result also agreed with Abdel-Sater and Mansour study in 2013, which showed statistically lowered serum calcium level while serum phosphorus showing non -significant elevation.[33] The present study results are also in agreement with Yang et al[34] whom showed that the Curcumin have the ability to improve the bone microarchitecture and bone mineral density in amyloid precursor protein/presenilin 1 (APP/PSI) transgenic mice. Moreover, Moon et al showed that Curcumin could stop osteoclastogenesis through suppression of RANKL activity completely in an in vitro study.[35] Further an in vivo studies conducted by Hussan et al and Cho et al showed that using Curcumin could limit bone loss, improve remodeling

and improve bone strength in osteoporosis animal models.[36,37] These previous in vitro and in vivo studies of Curcumin activity at osseous level could possibly be used to explain their significantly higher value than our control group.

Conclusion

The natural products extract, Curcumin and Nigella sativa oil may be systemically used for possibly enhancing bone response around orthodontic implant as reflected by lower serum calcium level as compared to control group.

Conflicts of interest

The authors report no conflicts of interest.

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