Effects of Oral Supplementation of Pomegranate Peel Extract on Some serum biochemical Parameters Related with Bone in Rabbit

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ABSTRACT

Background and aim: Pomegranate is a medicinal herb that can promote healing of periodontal tissue through differentiation of mesenchymal cells both in vivo and in vitro. Therefore, this study is to investigate the effect of oral supplementation of Punicagranatum L. peel extract on bone defect in rabbit.

Methods: Forty five male rabbits were divided into 3 groups; group 1; baseline group(5 rabbits) left without bone defect. Group 2; study group (20 rabbits) with bone defect model that received daily 1ml of oral supplementation of pomegranate peel extract (PoPx). Group 3; control group (20 rabbits) with bone defect model that received distilled water. Bone defect was done into facial plate of lower right central incisor. Blood biopsies by cardiocentesis at times (base line, 3h, 1, 3 and 7days) for estimation of serum calcium, phosphorous, and vitamin D levels.

Results: The results showed a significant increase in serum calcium and phosphorous levels only after 3 hours and 1 day of bone defect, in rabbits receiving water and rabbits receiving pomegranate peel extraction. Serum vitamin D level shows significant increase in all time intervals reaching maximum value after three days in rabbits receiving pomegranate peel extract, while no significant change was observed in rabbits receiving water.

Conclusions: Supplementation of pomegranate peel extract can increase vitamin D absorption, thus it may promote the bone healing process.

Keywords: Punicagranatum L. peel, surgical bone defect, biochemical activity, vitamin D, calcium, phosphorous.
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INTRODUCTION

Animal models have a big role in the generation of new knowledge in medical sciences, including periodontology. These experimental models have distinct advantages because they can reproduce in vivo cellular characteristics and reactions that occur in humans. Animal models of periodontal disease are particularly important in the development of the scientific basis for understanding the pathological processes [(1)].

Pomegranate (Punicagranatum L. Punicaceae; the common name has been derived from Latin words ponus and granatus), a seeded or granular apple, is a delicious fruit[(2)]. Pomegranate peels (PoP) have been characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit [(3)]. Topical application of a dentifrice containing pomegranate has a significant reduction of gingival bleeding in patients with periodontitis[(4)]

Research proved that pomegranate peel extract (PGPE) metabolites can directly modulate bone cell differentiation, leading to an improved resorption/formation ratio together with anti-inflammatory and anti-oxidative effects in the bone microenvironment[(5)]. The anti-inflammatory components of PoP, i.e., punicalagin, punicalin, strictinin A and granatin B significantly reduce production of nitric oxide and prostaglandin by inhibiting the expression of proinflammatory proteins [(6,7)]. Epithelialization, antioxidant effects, and characteristic biochemical properties are the usual features of the wound healing process that prevail in injured tissues. Topical administration of PoPx can be recommended for dead space wound, incisional and excisional wound models. Improved epithelialization, breaking strength and contraction of incised wounds, along with increased hydroxyproline content and breaking strength of granulated tissues, can be observed in the healing process of PoPx-treated wounds. In studies, oral administration of a 100 mg/kg aqueous extract of PoPx to Wistar rats and topical application of PoPx formulated with hydrophilic gel resulted in

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significant improvement in all wound models [8,9]. A recent study on isolated human neutrophils indicated that aqueous PoPx directly inhibited neutrophil myeloperoxidase activity and the enzymatic production of hypochloric acid from hydrogen peroxide at a 50 ng/ml concentration[10]. Thus, this study was carried to investigate the healing effect of forced oral administration of aqueous Pomegranate peel extract through analyzing various biochemical markers, such as Calcium(Ca), Phosphorous (P) and Vitamin (D) (Vit D) during experimental mandibular defect in rabbits.

MATERIALS and METHODS

Study Program

The study was carried out in Hawler Medical University, college of medicine, animal house. The work was undertaken on 45 apparently healthy male rabbits (aged 8-10 months, and weighing 1-1.2 Kg) randomly divided into three groups; the baseline group (5 rabbits) left without bone defect as (group 1), animals with surgical created bone defect model have been divided into rabbit groups (20 animals for each group, five rabbits for each time). The second group of pets (study group) orally received daily 1ml of ethanolic extract of Punica granatum L. peel extract, starting from seven days before surgically created a mandibular bone defect, and continue7 days post-operatively after bone defect, according to wound healing model. The third group (control group), their pattern with surgical created bone defect has been received 1 ml of distilled water. They were kept in cages, given water and fed with commercial food pellets. The study protocol was approved, and care of the animals has been taken as per standard rules.

Bone defect model

Animals were anesthetized with ketamine (40mg/kg) and xylazin (4mg/kg) body weight [11], a sulcular incision of 2 cm in length extend along the distolabial surface of the lower right central incisor to the distolabial surface of the lower left central incisor by using a # 15 Bard-Parker scalpel blade. A circular defect of 3 mm in diameter made in the mid labial area of alveolar bone of the lower right central incisor after full thickness flap reflection [12], the area was irrigated by 2ml of normal saline (0.9% NaCl). The incision was sutured by one stitch with (4/0) black silk to close the wound and replaced into the same previous position. The suture has been removed after (7) days. The cut has not been dressed, and no systemic or local antimicrobial agents have been used.

Pomegranate Peel Extract Preparation

About 500 g of punica peel from the fresh fruits have been obtained from a local shop were dried at 40 °C in an oven. The particles have been finely powdered in a mixture followed by filtration to remove largely remnants, then grounded by a mill then extracted in 80% of ethanol-water by maceration method. The extract then filtered two times by using Whitman paper No. (42). The filtrate was dried at 50 °C to get a fine dry powder that was prepared later as a solution that contains 100mg of punic peel extract in one ml of distilled water for the administration to the animal study group[13].

Animals of the second group received I ml concentration of (50mg/kg) [14] of aqueous extract of *Punicagranatum* L. peel extract orally. The dose of *Punicagranatum peel* extract was given once daily, starting from seven days before surgically created a mandibular bone defect, and continuing till the seventh day after bone defect form, according to the wound healing model. While the third group received distilled water (1ml) as placebo and served as control.

Blood sample collection

Five ml of blood have been collected cardiocentesis from each rabbit under subcutaneous general anesthesia [11], before surgical bone defect (0 day) and after surgical bone defect in the following time intervals (3h, 1, 3 and 7days). Blood samples have been centrifuged (3000rpm for 5 min at room temperature). The serum samples have been subsequently harvested, divided, and stored at -20 °C until they have been used for biochemical investigations including; estimation of serum calcium, serum phosphorous and serum vitamin D levels. Serum Calcium, phosphorous and vitamin D were quantitatively determined using a specific kit for Cobas C analyzer (Roche/Hitachi Cobas c systems) depending on a photometric method [15,16] and Elecsys Vitamin D total II assay [17, 18, 19].

Statistical analysis

Data were analyzed using the statistical package for the social science (SPSS, Statistical for Windows, version 20.0 Armonk, NY: IBM Corp). All data were expressed using descriptive statistic as the mean and standard deviation ± (SD). Statistical analysis using Paired sample t-test, a t- test is used when we wish to compare two means. A p - value of ≤ 0.05 was considered statistically significant, and (P value 0.01 and less) deliberate a highly significant difference.

RESULTS

Table (3-1), shows the mean concentration and standard deviation (±SD) for serum calcium (Ca), phosphorous (P), and vitamin D levels in rabbit's without receiving extract (received distilled water only) before and after surgically created mandibular bone defect. From the results, a

significant increase in serum calcium and phosphorous levels (concentration) has been found, three hours (11.76±0.27 and 9.20±0.48 respectively) and one day (9.75±0.31 and 7.79±0.39 respectively) after surgically created mandibular bone defect. The difference in the mean value scores with p value < 0.05 is statistically significant. Then serum calcium and phosphorous levels returned nearly to its baseline value in the next time intervals. The results showed that there was no expressive changes in serum vitamin D levels in rabbits that received distilled water after surgically created mandibular bone defect at all-time intervals, the difference showed no statistical difference.

Table (3-2), shows the mean and standard deviation (±SD) of serum calcium, phosphorous, and vitamin D levels in rabbits receiving pomegranate peel extract, before and after time intervals of creating mandibular bone defect. Serum Ca levels showed significant increase, three hours (12.93±0.53) and one day(11.65±0.69), then the degree returned nearly to their reference value (before creating bone defect) at the time interval three days and seven days after bone defect, the difference showed no statistical difference. The results also showed that serum phosphorous level significantly increased (7.17±0.061 and 6.90±0.136), 3 hours and one day after surgically creating bone defect respectively, then it decreased and returned nearly to its baseline value (5.58±0.23), three days after creating bone defect (p value more than 0.05).

A significant increase in serum vitamin D has been found, 3hours and 1day, reaching maximum (3.80±0.48) at time interval three days after creating bone defect in rabbits receiving pomegranate peel extract. The difference in the mean value scores with p value < 0.01 which is statistically a highly significant; finally decline at the 7 days intervals but still significantly higher than its value before creating bone defect.

Table (3-3) shows the comparison of mean value between study group and control group with time intervals after creating surgical bone defect in mandibular anterior teeth, was assessed by using Paired sample t-test. A non- significant difference in calcium and phosphorous levels were observed, after 3 hours and 1 day.

Results showed a significant difference at seven days' time interval after creating bone defect in relation to calcium level. On comparison between the two groups in relation to phosphorous level, significant difference has been found after (3 days and 7 days) of time intervals of bone defect, the difference in the mean value scores with p value < 0.05 which is statistically significant. Regarding serum vitamin D levels, comparison shows significant differences with all-time intervals.

Table 3.1: Intra control group comparison of mean concentration ±SD of serum Ca, P and Vitamin D levels each time interval after creating surgical bone in comparison to baseline data.

Parameters	Time intervals		Mean ± SD	SE	*t*-test	*P*-value
Calcium (mg/L)	before bone defect	baseline	9.18±0.27	0.275		
	after bone defect	3H	11.76±0.27	0.409	7.12	S
		1D	9.75±0.31	0.310	10.02	S
		3D	7.76±0.70	0.702	3.95	NS
		7D	7.64±0.55	0.557	3.76	NS
Phosphorous (mg/L)	before bone defect	baseline	5.47±0.67	0.103		
	after bone defect	3H	9.20±0.48	0.485	10.13	S
		1D	7.79±0.39	0.391	4.25	S
		3D	6.41±0.25	0.259	2.28	NS
		7D	6.15±0.33	0.337	3.70	NS
Vitamin D (IU)	before bone defect	baseline	2.33±0.30	0.305		
	after bone defect	3H	2.47±0.46	0.466	0.71	NS
		1D	2.30±0.39	0.391	0.122	NS
		3D	2.28±0.05	0.051	0.26	NS
		7D	2.20±0.30	0.300	0.46	NS

Table (3.2): Intra study group comparison of the mean concentration ± SD of serum Ca, P and Vitamin D levels throughout the study/ time interval in comparison to baseline data.

Parameters	Time intervals		Mean ± SD	SE	*t*-test	*P*-value
Calcium (mg/L)	before bone defect	baseline	9.18±0.27	0.275		
	after bone defect	3H	12.93±0.53	0.534	8.01	S
		1D	11.65±0.69	0.698	4.55	S
		3D	9.21±1.32	1.326	0.03	NS
		7D	9.12±0.11	0.115	0.22	NS
Phosphorous (mg/L)	before bone defect	baseline	5.47±0.67	0.103		
	after bone defect	3H	7.17±0.061	0.592	5.46	S
		1D	6.90±0.136	0.550	4.27	S
		3D	5.58±0.23	0.107	1.12	NS
		7D	5.46±0.24	1.072	0.43	NS
Vitamin D (IU)	before bone defect	baseline	2.33±0.30	0.305		
	after bone defect	3H	3.04±0.16	0.169	7.44	S
		1D	3.55±0.65	0.650	5.06	S
		3D	3.80±0.48	0.486	14.06	HS
		7D	3.77±0.13	0.132	5.85	S

Table3.3: Intergroup comparison between mean values of study group and control group regarding serum Ca, P and Vitamin D levels in rabbits with each time interval after creating surgical bone defect in mandibular anterior teeth.

Parameters/ Time interval		Study group mean ±SD	Control group mean ±SD	*P*-value
Calcium (mg/L)	3H	12.93±0.53	11.76±0.27	NS
	1D	11.65±0.69	9.75±0.31	NS
	3D	9.21±1.32	7.76±0.70	NS
	7D	9.12±0.11	7.64±0.55	S
Phosphorous (mg/L)	3H	7.17±0.061	9.20±0.48	NS
	1D	6.90±0.136	7.79±0.39	NS
	3D	5.58±0.23	6.41±0.25	S
	7D	5.46±0.24	6.15±0.33	S
Vitamin D (IU)	3H	3.04±0.16	2.47±0.46	S
	1D	3.55±0.65	2.30±0.39	S
	3D	3.80±0.48	2.28±0.05	S
	7D	3.77±0.13	2.20±0.30	S

DISCUSSION

The results of serum Ca and P in rabbits, that received distilled water (instead of pomegranate peel extract), showed that the level increased significantly, 3hours after bone defect creating. This elevation may be due to bone defect creating that causes bone cell destruction and releasing of cell contents (including Ca and P) into plasma [20, 21]. The values decreased (less than base values in relation to calcium level) at the next time intervals. This decrease may be due to bone repairing and remineralization that require calcium and phosphorous that supplied from the blood.

The results of this study showed that serum Ca and P in rabbits, that received pomegranate peel extract, increased significantly, 3hours and one day after bone defect creating. This increase is due to the same reason as in the case of rabbits receiving distilled water [21]. In the next time intervals, the values decreased and nearly returned to baseline values. These results are due to the increase in serum vitamin D, which was caused by the effect of pomegranate peel extract that may increase intestinal absorption of vitamin D. Thus, in turn, vitamin D can regulate both serum calcium and phosphorous levels [22]. The chief role of vitamin D is to maintain average blood levels of calcium and phosphorus. It helps the body absorb calcium, which forms and keep sound bones. Vitamin D is used alone or together with calcium to improve bone health and decrease fractures [23]. Vitamin D

declines calcium and phosphorus excretion in kidney [22, 20].

To rule out the impact of vitamin D metabolites; it was found that it had an important role in bone mineralization, these metabolites had effects on systemic calcium homeostatic mechanisms, which themselves impacted on bone. The lack of vitamin D results in hypocalcemia and hypophosphatemia [24].

CONCLUSIONS

According to the results of this study, conclusion has been drawn that oral Supplementation of pomegranate peel extract to rabbits can increase serum vitamin D that have an essential role in bone mineralization in case of bone defect creating, thus could be promoting the bone healing process.

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ألخلاصة

خلفية و اهداف البحث : الرمان, هو عشب طبي يمكن ان يعزز من شفاء أنسجة ما حول الاسنان من خلال التمييز التخصصي للخلايا (الوسيطة) على حد سواء, اكان مختبريا او داخل الجسم الحي. هدف هذه الدراسه هو التحقيق من تأثير تناول شراب مستخلص قشر الرمان على الارانب غير المصابه بالهشاشه بعد عمل جرح عظمي.

طرائق البحث : استخدم خمسه واربعون ارنبا ذكرا في الدراسة, وتم تقسيمهم الى ثلاث مجاميع : المجموعة الاولى (مجموعة الاساس)، تتكون من(5) ارانب ، بدون اصابة العظم بخلل,اما المجموعة الثانيه (مجموعة الدراسة) تتالف من(20) ارنبا اجريت لهم نموذج جرح عظمي(خلل) و تعالجوا بمستخلص الايثاني لقشر الرمان يوميا بمعدل (1 مل) عن طريق الفم . اما المجموعة الثالثة (مجموعه السيطرة) تتالف من (20) ارنبا, اجريت لهم جرحا عظميا و تم اعطاهم ماء مقطرا بدلا من مستخلص الايثاني لقشر الرمان. تم اجراء نموذج الخلل (الجرح) في العظم على اللوح الوجهي للقواطع الوسطية اليمنية السفلية.جمعت نمادج الدم عن طريق سحبها من القلب خلال فترات زمنية (0 ساعه، 3ساعه، 1 يوم، 3 يوم و 7 أيام) بعد اجراء خلل العظم.

النتائج: أظهرت النتائج المستحصلة من الدراسة ان هناك زيادة ملحوظة في مستويات الكالسيوم والفوسفور في مصل الدم للفترات الزمنيه (3 ساعات و 1 يوم) بعد اجراء الخلل العضمي عند الأرانب المعالجة بمستخلص الايثاني لقشورالرمان والارانب المعالجه بالماء .كما اظهرت النتائج ان هناك زياده معنويه في مستوى فيتامين د في مصل الدم لجميع الفترات الزمنية والتي تصل إلى أقصى قيمة بعد ثلاثة أيام من اجراء الخلل العضمي عند الأرانب المعالجه بمستخلص قشور الرمان ، في حين لم يلاحظ أي تغير معنوي لجميع الفترات الزمنية في الارانب المعالجه بالماء.

الاستنتاجات: ان استخدام مستخلص قشر الرمان له دور هام في زياده نسبه معادن العظام وذلك من خلال زياده نسبة فيتامين (د) في مصل الدم وبالتالي يمكن له دورفي دعم عمليه ترميم و شفاء الخلل العظمي.