Serum Creatinine and Creatine Kinase as an Indicator for Gingival Wound Healing in Rabbits by Supplementation of Pomegranate Seed Extract

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Abstract

Background: Pomegranate (Punica granatum) is an edible fruit that has been described as a medical and therapeutic functional food in the Middle East and the Mediterranean.
Objective: To investigate the use of serum creatinine (Cr) level and creatine kinase (CK) activity as indicators for gingival wound healing process rate in rabbits, supplemented on pomegranate seed extract (PSE).
Patients and Methods: A total of 45 rabbit males were used. They were divided into 3 groups; 5 rabbits as a baseline group that left without a buccal gingival wound. 20 rabbits (5 rabbits per each time interval) as a study group with buccal gingival wound with PSE supplementation, and another 20 rabbits (5 rabbits per each time interval) as a control group with gingival wound without PSE supplementation. A buccal gingival wound was created on the lower right central incisor, and the suture was removed after (7) days. Blood samples were collected for the baseline group and at time intervals; 3 hour, 1, 3, 7 days after creating the wound for both control and study groups to determine serum Cr and serum CK.
Results: Serum Cr and CK significantly increased in all time intervals after gingival wounds, in the control group compared with baseline values. Whereas the levels significantly increased in rabbits receiving PSE at intervals of 3 hours, 1 and 3 days after gingival wound, they returned to the baseline values, seven days after gingival wound incision.
Conclusion: Serum Cr and CK increase in the buccal gingival wound, while oral supplementation of PSE can decline them to the baseline value after a period of time, therefore these parameters can be used as indicators for gingival wound healing rate.
Keywords: Punicagranatum seed extract, gingival wound healing, creatinine, creatine kinase.

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Introduction

Oral cavity wounds can be caused by many factors, such as; surgery, trauma, and oral disease[1]. Many medicines such as; antibiotics, corticosteroids, Non-steroidal anti-inflammatory drugs (NSAIDs), and disinfectants, such as chlorhexidine have been used for speeding up the healing process rate of oral wounds[2]. However, these medicines have many side effects, such as gastrointestinal damage, discoloration, dysgeusia, and excessive sensitivity in the oral mucosa [3]. Natural products can replace these medicines [4,5]. Indeed, some plants have more healing effects and fewer side effects than these medicines [6,7].

Pomegranate (*Punica granatum*) is one of the oldest fruits that have been known in the Middle East [8]. All parts in PG are rich in some biologically active compounds, such as phenolics and flavonoids. Therefore, it has been described as one of nature's fruits that has been used in the medical field [9-11].

Recently, studies have shown that pomegranate can treat many cases, due to its biological activities, such as anti-inflammatory, antibacterial, and antioxidant [11,12]. Polyphenols, such as tannins, have the primary responsibility for its treating effects of PG, especially its wound healing potency [13, 14]. PG extracts are known to induce proliferation, collagen production, and fibroblast migration [15-17]. Prasad and Kunnaiah, 2014 reported that PG be used to treat periodontal disease and periodontium wound healing [18].

Creatinine is a non-proteinic nitrogen compound formed by a non-enzymatic dehydration reaction followed by cyclization of creatine in muscle. Creatinine is found in plasma, serum, and urine [19]. Serum creatinine is a reliable marker of muscle mass.

Creatine, the precursor of creatinine, provides energy to muscle cells [20]. Creatine is produced primarily in the kidney and liver, then can transport to muscle and brain where phosphorylated and stored as an energy source; phosphocreatine [19,20].

Creatine kinase (CK) or creatine phosphokinase (CPK) is an enzyme that catalyzes the phosphorylation of creatine [20,21] . Lombao et al, published that, serum CK concentration is an important marker of surgical muscle injury and is readily released during muscle injury [22]. Creatine kinase (CK) is an enzyme that is used to screen muscle injury among athletes and sports players [23]. This is primarily due to that CK has a relative predominance in the skeletal muscles, and is readily released during muscle injury [24]. Studies have reported that nonsurgical periodontal therapy can reduce periodontal inflammation and serum CK levels [25,26].

Therefore this study was designed to evaluate using serum creatinine (Cr) and creatine kinase (CK) as an indicator for buccal gingival wound healing in rabbits, by supplementation of Pomegranate seed extract.

Patients and Methods

Study diagram

This study was performed in Hawler Medical University, college of dentistry, basic science department. A total of 45 healthy rabbit males (1.3-1.5 Kg) with the age of 6-7months were used and divided into

3 groups; a baseline group (5 rabbits) that was left without creating an oral wound, while for the other rest animals (40 rabbits), an oral incision wound was done for them. They were divided into two groups (20 animals for each group, five rabbits for each time); study group which orally received(100mg/kg/day) aqueous PSE, starting from 2 weeks before the surgical gingival wound and continued for 7 days after the operation, according to wound healing model. While the control group was with the same pattern of the buccal gingival wound only, it has been left without treatment. They were kept in cages, in the animal house of the college of medicine, given water, and fed with commercial food pellets.

Surgical procedure

This part was done by a specialized dentist. All the rabbits were anesthetized using 40mg/kg ketamine and 4mg/kg xylazine[27]. A wound was made, using a # 11 Bard-Parker scalpel blade, through the marginal gingival. The incision starts within the gingival sulcus, and is extended toward the tooth surface to the level of the alveolar crest. To close the wound and replace in the same previous position, a stitch with (4/0) black silk was used to suture the wound margins. The suture was removed (7) days after surgical creating a wound and suturing.

Pomegranate seed Extract Preparation

The pulps of pomegranate were pressed and the juice was removed, the seeds were taken and dried, then powdered by a grinder. 200g of the powder was weighed and dissolved in 600ml of 70% ethyl alcohol in water, then stored at room temperature for 72 hours. The mixture was filtered, and the extract was obtained after evaporating the solvent using a rotary evaporator at 50°C. 0.1 g of the extract powder was suspended in 1 ml of distilled water [28], then this suspension was orally administered to the study group.

Collection of Blood Sample

The rabbits were anesthetized with subcutaneous injection of 40mg/kg of ketamine and 4mg/kg of xylazine after each experiment [27], and then blood samples were obtained from the rabbits by cardiocentesis. Blood was taken for the study and control groups after creating an incision at time intervals; 3hurs, 1,3, and 7 days. All the samples were centrifuged at3000rpm for 10 minutes around 25°C. Serum samples were stored at -20 °C until the chemical analysis. Serum Creatinine was determined by a specific Biolabo company kit [29,30]. While Serum CK activity was determined using a specific Biolabo company kit depending on a colorimetric method [31,32].

Statistical analysis

Version 22 was used for the data analysis. The data of this study was described by the mean with the standard deviation ± (SD). A t-test was used for comparing two means. P-value of 0.05 or less was regarded as a statistically significant difference.

Results

The serum Cr and CK activities in the baseline group and Control group are shown in table (3-1). From the results, a significant increase in serum Cr levels and serum CK activities have been found, three hours(1.05±0.16, 187.75±9.17 respectively), one day (1,78±0.18, 244.34±12.61 respectively) and three days (2.25±0.23, 273±13.35 respectively) after gingival wound

incision comparing with the baseline values. Then these increased values declined 7 days after incision (1.55±0.21 and 202.56±11.72 respectively), but still significantly higher than its baseline value (0.71±0.14, 165.22±8.68 respectively).

The serum Cr levels and serum CK activities in rabbits of the study group at time intervals after creating gingival incision are shown in table (3-2). The results indicated a ranging from 8-77 years, the median age was 45.5 years, male: female ratio was 1.07:1. significant increase in serum Cr levels and CK activities, three hours (0..10±0.13, 183±8.44 respectively), one day (1.65±0.20, 216±11.39 respectively), and three days (1.33±0.18, 190.37±12.35 respectively) after creating gingival incision wound compared with the baseline values, then they declined at the time interval; seven days after suture removal, and they returned close to its baseline values before incision.

Table (1): Serum Cr levels and CK activities (mean±SD) in rabbits receiving distilled water (Control group) before (baseline) and after time intervals of creating gingival incision wound

Parameters	Time intervals		Mean ± SD	*P*-value
Serum Cr. (mg/dL)	Before incision	Baseline	0.71±0.14	
	After incision	3H	1.05±0.16	S
		1D	1.78±0.18	S
		3D	2.25±0.23	S
		7D	1.55±0.21	S
Serum CK (IU/L)	Before incision	Baseline	165.22±8.68	
	After incision	3H	187.75±9.17	S
		1D	244.34±12.61	S
		3D	273±13.35	S
		7D	202.56±11.72	S

*S: significant, H: hour, D:day

Table (2): Serum Cr levels and CK activities (mean±SD) in baseline group and study group (rabbits receiving pomegranate seed extract at time intervals after creating a gingival incision wound

Parameters	Time intervals		Mean ± SD	*P*-value
Serum Cr. (mg/dL)	Before incision	baseline	0.71±0.14	
	After incision	3H	0.10±0.13	S
		1D	1.65±0.20	S
		3D	1.33±0.18	S
		7D	0.78±0.15	NS
Serum CK (IU/L))	Before incision	baseline	165.22±8.68	
	After incision	3H	183±8.44	S
		1D	216±11.39	S
		3D	190.37±12.35	S
		7D	176.50±10.47	NS

* NS: non-significant, S: significant, H: hour, D: day

Discussion

The results showed a significant increase in serum Cr level and CK activity after gingival wound incision, but the values in rabbits receiving PSE (study group) declined seven days after suture removal, and they returned close to their baseline values. This decline in the serum Cr value and CK activity may be related to the effect of the PSE which contains antioxidant and anti-inflammatory agents that reduce the formation of nitric oxide and Prostaglandin E2 (PGE2) [33,34]. Therefore gingival and periodontal tissue damage will decrease, and Cr and CK release into the blood also will decrease [34].

Reactive oxygen species (ROS) damage cells and tissues; hence they are bad for wound healing and they peroxidize lipids, inactivate enzymes, and break DNA down [35].

Pomegranate parts contain powerful antioxidants shown to improve human health. The antioxidant and anti-inflammatory properties of pomegranate fruits, which can accelerate the wound healing process, can be attributed partly to vitamin C and vitamin E that pomegranate contains. Besides, tannins and polyphenols like ellagic acid, gallic acid, and coumaric acid are found in pomegranate seeds [36-39]. The presence of polyphenols (such as gallic acid) and quercetin in pomegranate can improve muscular strength and recovery [40-42].Researchers investigated the effects of oral administration of pomegranate peel (PPE) extract on the healing of surgically induced full thickness of the gingival wound. Their results showed that oral administration of PPE can stimulate healing properties in gingival wounds, in the rabbit model by controlling free radicals and lipid peroxidation [43].

The observation of the study was in the same line as that of Chraf *et al*, who concluded that Pomegranate juice seems to have significant effects on serum creatinine, muscle damage parameters (such as creatine kinase), and C-reactive protein [44]. Our finding is agreement with Ammar *et al*, who found that both serum CK and Lactate dehydrogenase (LDH); the biomarkers for muscle damage during exercise, were reduced after pomegranate supplementation [42].

The result of the present study is in the same line as the result of Alshail *et al*, who found that increases in the periodontal tissues are associated with increased serum CK levels. Thus, they concluded that the increase in serum CK level might indicate a compromised oral health status [45]. The results are in the same direction as Cintra et al results, who found that increase in serum creatinine is related to gingival damage due to oral infections [46]. Also, the result of the study is in agreement with Yoshihara *et al* finding, who observed relation of gingival and periodontium tissue damage with creatinine level [47].

Conclusions

From the results, it can be concluded that gingival wound healing can be accelerated by PSE supplementation, and this phenomenon was in line with the reduction in serum Cr and serum CK activity, thus these parameters can be used as indicators for gingival wound healing rate.

Recommendations

Testing the other parts of pomegranate fruit extracts such as the peel for its potency in speeding up the rate of gingival wound healing and the use of both serum Cr and CK as indicators in this case.

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Ethical clearance: The study was approved by the academic ethical committee, College of Dentistry, Hawler Medical University.

Conflict of interest: Nill

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