Evaluation of Some Serum Antioxidants in Mandibular Bone Defect Healing in Rabbits Orally Supplemented with Pomegranate Peel Extract

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Abstract

Background: Pomegranate (PG) fruit (punicagranatum L, Punicaceae contains many bioactive substances that play important role in the treatment of a variety of medical problems. Among PG parts, it was observed that the peel had the most potent antioxidant activity.

Objective: To evaluate some serum antioxidants levels in mandibular bone injury healing in rabbits orally supplemented with pomegranate peel extract (PPE).

Patients and Methods: Forty-five male rabbits (1-1.2 kg) were used and they were divided into 3 groups; the baseline group consisted of 5 rabbits without creating mandibular bone defects. 20 rabbits were used as a study group with creating mandibular bone defects and they received orally 1ml/day of PPE. Another 20 rabbits were used as a control group with creating mandibular bone defects without receiving PPE. For each rabbit of both study and control groups, a circular defect of 3 mm in diameter was made in the mid labial area of the alveolar bone of the lower right central incisor. Blood samples were taken from the baseline group and both the study and control groups at time intervals; 3hrs, 1, 3, and 7days after bone defect creation, for estimation of serum albumin (Alb), bilirubin (Bili), zinc (Zn), and magnesium (Mg) levels.

Results: Serum Bili levels significantly increased 1and 3 days after bone defect creation in both study and control groups, while the levels returned back to baseline in a study group, 7 days after the operation. Serum Alb, Zn, and Mg levels showed a significant decrease in study and

control groups, 1 day and 3 days after the operation, while the levels of these parameters returned back to baseline, in a study group, 7 days after bone defect.

Conclusion: Supplementation of PPE can affect serum antioxidants and this was accompanied by the increase in the bone healing rate process.

Keywords: Pomegranate peel, mandibular bone defect, albumin, bilirubin, divalent cations, antioxidant

Introduction

Pomegranate (PG) was found to have anti-oxidant, anti-inflammatory, and antibacterial activities [1-4]. It was observed that the PG biological activity is mainly due to its antioxidant potency coming from anthocyanins and tannins [5,6]. Almost 26%–30% of PG fruit is the peel which has the highest anti-oxidant activity due to its content of a large number of polyphenols such as flavonoids, hydrolysable tannins and punicalagin[8,9].

The PG peel was used as a treatment for many medical cases, such as osteoporosis, which is a chronic disease which is related to bone remodeling disturbance resulted in progressive bone loss due to the presence of oxidation stress in the osteoporosis pathogenesis[10]. Spilmont et al, investigated that whether the consumption of PPE could limit the process of osteopenia[11]. PPE was formulated as a topical gel and was examined for its wound healing property against skin excision wounds. The topical application of this PPE formulated resulted in significant improvement in the wound model[12]. The skin extract of PG was studied for its wound healing potency in the rat excision wound model, it was found that PG promotes significant wound healing in rats[13].

Bone is continuously remodeled by the roles of both osteoblasts and osteoclasts[14]. Disturbance in bone remodeling may be caused by an increase in resorption activity over the bone-formation rate or by low bone turnover [15]. It was found that polyphenolic compounds can affect osteoporosis[16,17], inhibit osteoclast differentiation18 and stimulate osteoblast formation[19].

This study was designed to evaluate some serum antioxidants (albumin, bilirubin, Zn and Mg) in mandibular bone wound healing in rabbits orally supplemented with PPE.

Patients and Methods

Animals

This study was performed in Hawler Medical University, College of Medicine, animal house and College of Dentistry, Department of basic sciences. The work was carried out on 45 healthy male rabbits (aged 8-10 months, and weighing 1-1.2 Kg), they were divided into three groups; a baseline group consisting of 5 rabbits without mandibular bone defect creation. The study group consisting of 20 animals (5 rabbits for each time interval) orally received 1ml/day ethanolic PPE, starting from seven days before surgically created a mandibular bone defect, and continuing till 7 days after the mandibular bone defect operation, according to wound healing model. The third group; the control group consisted of 20 animals (5 rabbits for each time interval), they received 1ml/kg of distilled water instead of PPE. All

the animals were fed with commercial food pellets and given water according to standard care rules.

Mandibular bone defect operation

Rat IL-1B ELISA kit for estimation of serum interleukin-1beta (IL-1β), KOMA BIOTECH INC.USA Aloe Vera's mature, safe and fresh 98% (gel) Chlorhexidine 2% (Periocare) gel (Kin company-Spanish).

Animals, Experimental studies and sampling

This part was performed by a specialized dentist. After anesthetizing the animals with (40mg/kg) ketamine and (4mg/kg) xylazin [20], and by using a # 15 Bard-Parker scalpel blade, a 2 cm in length sulcular incision was extended along the distolabial surface of the lower right central incisor to the distolabial surface of the lower left central incisor. In the mid-labial area of the alveolar bone of the lower right central incisor, a circular defect of 3 mm in diameter was made after full-thickness flap reflection[21], 2ml of normal saline was used to irrigate the area. To close the wound and replaced in the previous position, the incision was sutured by one stitch with (4/0) black silk; the suture was removed after 7 days without using antimicrobial agents.

Preparation of Pomegranate Peel Extract

Rafraf method was used to prepare PPE[22]. 500 g of Punica peel from the fresh fruits was obtained and dried in an oven at 40 °C. The particles were powdered finely by a grinder and filtrated to remove the remnants, then extracted in 80% ethanol-water by maceration method. Using Whatman filter paper No.42, the extract was filtered two times. The filtrate was dried at 50 °C, then 100mg of dry powdered Punic peel was suspended in 1ml of distilled water,.Finally, this suspension was administrated to the animals in the study group.

1 ml of 50mg/kg/day of the PPE suspension was orally supplemented to the rabbits of the study group. This supplementation started 7 days before the surgical operation, and continued until 7 days after the operation (according to the wound healing model)[23], while the control group orally received 1ml distilled water /day instead of PPE suspension.

Blood collection

Under subcutaneous general anesthesia and through cardiocentesis, 5ml blood was collected from each rabbit [20] of the baseline group and both study and control group at time intervals; 3h, 1, 3, and 7days after the operation. The blood samples were centrifuged for 5 min at 3000rpm, then the serum was separated and stored at -20 °C until chemical analysis, which included; determination of serum Alb[24], Bili[25] , Zn[26]and Mg[27] levels. These parameters were calorimetrically determined using specific kits.

Statistical analysis

The statistical package for the social science (SPSS, Statistical for Windows, version 20.0 Armonk, NY: IBM Corp) was used for data analysis. The data of this study was described by the mean with the standard deviation ± (SD). A paired sample t- test was used for comparing two means. The difference in the mean value scores with p-value < 0.001 for serum Bili, Alb and Mg and p-value < 0.05 for serum Zn are statistically significant.

Results

Table (1) shows the levels of serum Alb, Bili, Zn, and Mg in rabbits receiving PPE (study group) before and after surgically created mandibular bone defect. The results indicate a significant decrease in serum Alb, Zn, and Mg levels, one day (2.84±0.32, 82.55±4.82, and0.857±0.121 respectively) and three days (3.07±0.36, 80.34±4.56, and 0.876±0.145 respectively) after surgically created mandibular bone defect in comparison to baseline values. While 7 days after mandibular bone defect creating, the serum levels of all these parameters raised nearly to their baseline values. The results showed that there was a significant increase in serum Bili levels in rabbits receiving PPE, three hours and one day after a surgically created mandibular bone defect in comparison to the baseline value. While the Bili levels will retune back nearly to its baseline value, by 7th day after mandibular bone defect creation.

Table (1): Comparison of the mean concentration ± SD of serum albumin, bilirubin, zinc and magnesium levels (each time interval after creating surgical bone) in a study group to baseline data

Parameters	Groups	No. of Rabbits	Mean	Std. Deviation (± SD)	P-Value
Serum Albumin (g/dl)	Baseline	5	3.78	0. 32	
	3H	5	3.64	0.37	NS
	1D	5	2.84	0.32	S
	3D	5	3.07	0.36	S
	7D	5	3.75	0.37	NS
Serum Bilirubin (mg/dl)	Baseline	5	0.411	0.040	
	3H	5	0.462	0.033	S
	1D	5	0.473	0.034	S
	3D	5	0.431	0.038	NS
	7D	5	0.417	0.032	NS
Serum Zinc (ug/dl)	Baseline	5	103.63	5.22	
	3H	5	100.35	5.76	NS
	1D	5	82.55	4.82	S
	3D	5	80.34	4.56	S
	7D	5	102.85	6.11	NS
Serum Magnesium (mg/l)	Baseline	5	3.31	0.33	
	3H	5	3.05	0.34	NS
	1D	5	2.34	0.36	S
	3D	5	2.61	0.34	S
	7D	5	3.29	0. 31	NS

*H: Hour, D: Day, S: Significance, NS: Non-Significance

Table (2) shows the levels of serum Alb, Bili, Zn, and Mg in rabbits receiving distilled water (control group), before and after time intervals of creating a mandibular bone defect. Serum Alb, Zn, and Mg levels showed a significant decrease throughout all time intervals after bone defect creation in comparison to baseline values. The results also showed that serum Bili level significantly increased at all time intervals after creating a bone defect.

Table (2): Comparison of mean concentration ±SD of serum albumin, ilirubin, zinc and magnesium levels (each time interval after creating surgical bone) in control group to baseline data

Parameters	Groups	No. of Rabbits	Mean	Std. Deviation (±SD)	P-Value
Serum Albumin (g/dl)	Baseline	5	3.78	0.40	
	3H	5	3.58	0.35	NS
	1D	5	2.75	0.31	S
	3D	5	2.93	0.37	S
	7D	5	3.16	0.32	S
Serum Bilirubin (mg/dl)	Baseline	5	0.411	0.037	
	3H	5	0.473	0.035	S
	1D	5	0.461	0.035	S
	3D	5	0.452	0.032	S
	7D	5	0.445	0.030	S
Serum Zinc (µg/dl)	Baseline	5	103.63	5.22	
	3H	5	103.17	5.36	NS
	1D	5	83.32	5.02	S
	3D	5	78.77	4.88	S
	7D	5	86.47	5.95	S
Serum Magnesium (mg/dl)	Baseline	5	3.31	0.33	
	3H	5	2.94	0.38	NS
	1D	5	2.23	0.34	S
	3D	5	2.39	0.37	S
	7D	5	2.52	0.33	S

*H: Hour, D: Day, S: Significance, NS: Non-Significance

Table (3) shows the comparison of mean ±SD value between the study group and control group with time intervals after creating a surgical bone defect in mandibular anterior teeth (it was assessed by using Paired sample t-test). Significant differences in all the parameters levels were found between the study group and control group, only after 7days after bone defect creation, while non-significant differences in the parameters at all the other time intervals were observed.

Table (3): Inter group comparison between mean ±SD values of study group and control group regarding serum albumin, bilirubin, zinc, and magnesium levels in rabbits with each time interval after creating surgical bone defect in mandibular anterior teeth

Parameters	Time interval Hour (H) Day (D)	Study group (mean ±SD)	Control group (mean ±SD)	*P-value*
Serum Albumin (g/dl)	3H	3.64 ± 0.37	3.58 ± 0.35	NS
	1D	2.84 ± 0.32	2.75 ± 0.31	NS
	3D	3.07 ± 0.36	2.93 ± 0.37	NS
	7D	3.75 ± 0.37	3.16 ± 0.32	S
Serum Bilirubin (mg/dl)	3H	0.462 ± 0.033	0.473 ± 0.035	NS
	1D	0.473 ± 0,034	0.461 ± 0.035	NS
	3D	0.431 ± 0.038	0.452 ± 0.032	NS
	7D	0.417 ± 0,032	0.445 ± 0.030	S

Serum Zinc (µg/dl)	3H	100.35 ± 5.76	103.17 ± 5.36	NS
	1D	82.55 ± 4.82	83.32 ± 5.02	NS
	3D	80.34 ± 4.56	78.77 ± 4.88	NS
	7D	102.85 ± 6.11	86.47 ± 5.95	S
Serum Magnesium (mg/dl)	3H	3.05± 034	2.94±0.38	NS
	1D	2.34±0.36	2.23±0.34	NS
	3D	2.61±034	2.39±0.37	NS
	7D	3.29±0.31	2.52±0.33	S

* S: Significance, NS: Non-Significance

Discussion

Throughout The physiological role of antioxidants is to prevent damage to cellular components by free radicals[28]. Antioxidants are two types; small organic molecules and large molecules[29].

The results of this study indicated that serum Alb, Zn, and Mg levels decreased after surgically created mandibular bone defect, however daily oral administration of pomegranate peel extract can modulate these parameters levels, seven days after bone defect creating, while in distilled water administration group, the levels were still low, thus mandibular bone defect creating process resulted in a decrease in some serum antioxidants variables (such as albumin) and some metal ions (such as Zn and Mg) that have a vital role in immune and in controlling oxidative stress systems[30], while PPE has potency to increase these important serum antioxidants thereby speeding up the rate of bone healing process.

The features of the wound healing process that can be observed in injured tissues are epithelialization, antioxidant effects, and some biochemical characterizations [12]. It was published that, PPE can regulate the differentiation of bone cells, leading to an improvement in resorption to formation ratio, anti-inflammatory effects, and anti-oxidative potency in the bone microenvironment[31]. Oral administration of aqueous PPE extract to rats resulted in the healing of wound models [32]. The study of Shishehbor et al, 2016 showed that supplementation of pomegranate juice seems to improve some inflammatory markers and to increase plasma antioxidants status in type 2 diabetes[33]. The findings of Amri *et al*., 2017, showed the neuroprotective effects of PG extracts, and they explained the mechanisms throughout stimulating of some serum antioxidants [34]. Al-Olayan *et al*, 2014, studied the effects of PG consumption on some serum markers of oxidative stress. Their results showed that PG juice caused a significant elevation in serum antioxidants and a significant reduction in lipid peroxidation and nitric oxide [35].

It was found that the most important explanation of the positive effects of albumin, its antioxidant potency [36,37]. The antioxidant properties of albumin are associated with its structure. This is due to its content of multiple ligand-binding capacities for trapping free radicals [38,39]. Another explanation for the antioxidant properties of albumin was its ability to bind bilirubin,

lipids and homocysteine. The serum albumin-bilirubin complex can inhibit lipid peroxidation [39,40].

Serum albumin can regulate the cellular Glutathione (GSH) level, thus protecting cells from oxidative stress [41]. The Cys34 residue in albumin acts as a free radical scavenger, thus it has the ability for trapping reactive oxygen species (ROS) and reactive nitrogen species (RNS)[42].

In the present study, serum bilirubin increased after surgically created mandibular bone defect, however daily oral administration of PPE could modulate this parameter level, seven days after bone defect creating, while in distilled water administration group, the levels were still high.

Bilirubin is a product of hemoglobin degradation, it is known as an endogenous antioxidant. Bilirubin is produced by the reduction of biliverdin, then bilirubin can be oxidized by ROS and return back to biliverdin (a reversible process). In the body, bilirubin is a strong antioxidant and mostly can act against peroxyl radicals[43-45].

Zn and Mg are involved in the mechanisms of antioxidant defense[46]. Zn metal participates in the controlling of chronic inflammatory cases through reducing the inflammatory cytokines. Zn also takes part in the formation of the antioxidant enzymes, thus reducing the oxidative stress[30]. Marreiro et al, 2017 demonstrated that Zn took part in the structural integrity of superoxide dismutase (SOD), thus activating SOD which can reduce the toxicity of ROS throughout converting superoxide radicals to hydrogen peroxide and oxygen[47]. Zn is also a co-factor for many enzymes in the antioxidant defense system [48]. Zn can affect the gene expression of the enzyme glutamate-cysteine ligase; an important enzyme that regulates glutathione de novo synthesis, thus this is another mechanism by which Zn acts as antioxidant[49].

Studies have found that oxidative-inflammatory stress has been related to hypomagnesemia in Heart Disease patients[50,51]. In obese individuals, Mg deficiency participates in the developing oxidative stress, as Mg has a role as an antioxidant, it acts as a cofactor for many enzymes, that maintain cell membrane integrity and stability, thus reducing the effects of oxidative stress[52]. The study of Vida et al, 2020 revealed that Mg has the main role in regulating immune function and in decreasing oxidative stress[53]. The participation of Mg in the mechanisms of antioxidant defense is associated with glutathione synthesis[54]. A positive correlation exists between the concentration of Mg ions and the level of GSH in human blood [54,55]. Two enzymes that are Mg ion-dependent,catalyze GSH synthesis; these enzymes are gamma-glutamylcysteine synthetase and glutathione synthetase[56]. Shcharbina, 2008, found that Mg has an antioxidant effect in patients with stroke[57]. The possible mechanism of serum magnesium anti-oxidative action is attributed to its ability to inhibit lipid peroxidation catalyzed by Fe ions, and the supporting of the level of restored glutathione in the cell[58].

Conclusions

It can be concluded that PPE has the ability to increase the serum antioxidant potency to control the oxidative stress which resulted

from mandibular bone injury, thus increasing the rate of the healing process.

Recommendations

Evaluation of serum levels of other antioxidants and antioxidant capacity in the alveolar bone defect in rabbits orally supplemented with pomegranate peel extract, to confirm our results and conclusions.

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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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