Effect of Oral Supplementation for Rabbits of Pomegranate seed Extract on Some Serum Biochemical Parameters in Relation to oral Inflammation, oxidative stress, and wound healing

Rezan Majeed Omer, MSc, PhD. [(1)]
Dilyar Ahmed Baban, MSc, PhD. [(2)]
Bakhtiar Mohyadeen Ahmed, MSc, PhD.[(3)]

ABSTRACT

Background: Pomegranate (punicagranatum L, Punicaceae), is an edible fruit consumed around the world. The edible part of pomegranate is rich in compounds that possess antioxidant and anti-inflammatory activities. The aim of this study is to investigate the antioxidant; anti-inflammatory and gingival wound healing effects of Punicagrantum L. seed extract oral supplementation in rabbit.

Methods and Methods: Forty five male rabbits were divided into 3 groups, base line (5 rabbits) left without buccal gingival wound as(group 1),study group, 20 rabbits (group2) with buccal gingival wound treated with ethanolic extract of Punicagranatum L. seed extract and control, 20 rabbits (group 3) with buccal gingival wound only. Buccal gingival wounds were created on lower right central incisor and sutured removed after (7) days. Blood biopsies by cardiocentesis were collected at times (0, 3h, 1, 3 and 7days) for estimation of serum alkaline phosphatase activity, serum total proteins, and serum uric acid.

Results: The results showed a significant increase in serum Alkaline phosphatase, total proteins and uric acid in all time intervals after buccal gingival wound, in rabbits receiving water, while their levels increased significantly only at time intervals of 3 hours and 1 day after gingival wound, in rabbits receiving pomegranate seed extraction.

Conclusions: It has been concluded that oral Supplementation of pomegranate seed extract in rabbits can expedite the rate of healing of gingival wound.

Keywords: Punicagranatum L. seed extract, gingival wound, biochemical activity, healing process, alkaline phosphatase, total protein, uric acid. **(Received: 12/12/2018; Accepted: 25/1/2019)**

INTRODUCTION

Inflammation is a protective biological response that involves blood vessels, immunological cells, and inflammatory mediators [(1)]. Among the mostimportant inflammatory mediators, tumor necrosis factor-α (TNF-α) plays important role in inflammation by regulating the release of other mediators. It is also involved in chemotaxis, especially of neutrophils, and induces the expression of a range of receptors that contribute to amplifying the inflammatory response. The excessive and chronic release of TNF-α was implicated in a variety of pathological conditions such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, heart valve disease, and sepsis[(2)].

Natural compounds have been widely studied as potential anti-inflammatory drugs in search for novel therapeutic options that may cause fewer adverse effects [(3, 4)]. Pomegranate (Punicagranatum L., Punicaceae) has been suggested to possess wound-healing, antimicrobial, and antioxidant properties [(5, 6)].

(1) Lecturer, Oral Biochemistry, College of Dentistry, Hawler Medical University, Erbil, Iraq.
(2) Lecturer, Periodontology, College of Dentistry, Hawler Medical University, Erbil, Iraq.
(3) Assist. Prof., Oral & Clinical Biochemistry, College of Dentistry, Hawler Medical University, Erbil, Iraq.

Pomegranateis a long lived and drought tolerant plant. Arid and semiarid zones are well- known for growing pomegranate trees. However, pomegranate was categorized as a berry, but it belongs to its own botanical family, *Punicaceae*. The only genus is *Punica*, with one predominant species called *P. granatum* [(7)]. Pomegranate (punicagranatum L, Punicaceae), is an edible fruit consumed around the world. It is well documented that the edible part of pomegranate is rich in compounds that possess antioxidant and anti-inflammatory activities [(8)]. Almost all parts of a pomegranate (*Punicagranatum*Linn.) have well known as antioxidant activity [(9)]. Pomegranate and the selected chemical constituents isolated from juice, peel, and seed have been found to have an excessive range of effects: (i) inhibition of Cyclooxygenase-2 (COX- 2) expression and ultimately eicosanoid biosynthesis [(10)]; (ii) synergistic suppression of inflammatory cytokine expression [(11)]; (iii) inhibition of Matrix metalloproteinase (MMP) [(12)]. For that, the study aims to know the healing effect of forced oral administration of local pomegranate seed extract, and their efficacy on wound healing by analyzing various biochemical markers, such as Alkaline phosphatase (ALP), total protein (TP), and uric acid (UA) in serum rabbits.

MATERIALS AND METHODS

Study diagram

The study was carried out in Hawler Medical University, college of medicine, animal house. The work has been taken on apparently healthy 45 rabbits male (aged 6-7months, and weighing 1.3-1.5 Kg) randomly divided into three groups. The group (5 rabbits) left without oral incision wound as a baseline group; animals bearing a given wound have been divided into two groups (20 animals for each group, five rabbits for each time). The second group of pets (study group) orally received (100mg/kg) of aqueous extract of Punicagranatum L seed daily, starting from 2 weeks before the surgical gingival wound continue7 days post-operatively, according to wound healing model. The third group (control group), their pattern with buccal gingival wound only has been left without treatment. They were kept in cages, given water and fed with commercial food pellets.

Surgical procedure

Animals were anesthetized with ketamine (40mg/kg) and xylazin (4mg/kg) body weight [13]; wound was made through the marginal gingiva. The injury was made using a # 11 Bard-Parker scalpel blade. The incision began within the gingival sulcus, and it extended obliquely toward the tooth surface to the level of the alveolar crest. Wounds were made in the tissues on the labial aspects of the teeth of the lower right central incisor and extend through the interproximal area. Wound margins were 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.

Pomegranate seed Extract Preparation

The seeds of pomegranate have been obtained after pressing the pulps to remove the juice. The seeds dried in a shed then grounded to a fine powder by a grinder. About 400g of the fine was mixed with 1200ml of 70%ethanol in distilled water and kept for three days at room temperature. After the filtration of the extract, the solvent was removed by rotary evaporator at 50˚Cto obtain the extract. About 100mg of the powdered extract was suspended in 1 ml of distilled water [14] and administered to animals of the study group

Blood sample collection At the end of each experimental work, blood were drawn cardiocentesis from each rabbit, under general anesthesia with subcutaneous injection of xylazin (4mg/kg) and ketamine (40mg/kg)[13], before incision (0 day) and after wound incision in the following time intervals(3h, 1, 3 and 7days). Blood samples have been centrifuged (3000rpm for 5 min at room temperature). Serum samples were subsequently harvested, divided and stored at -20 °C for biochemical investigations**.** Serum ALP was quantitatively determined using a specific kit for combat analyzer (Roche/Hitachi Cobas c systems) depending on a colorimetric method described by King and Armstrong, modified by Hausamen et al., [15]. Serum TP was quantitatively determined using a specific kit for combat analyzer (Roche/Hitachi combat systems) depending on a colorimetric assay described [16]. Serum UA was quantitatively determined using a specific kit for combat analyzer (Roche/Hitachi combat systems) depending on enzymatic colorimetric test described and modified by Town et al., [17].

Statistical analysis

Data from this study were analyzed using the statistical package for the social science (version 22). 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 or less) was considered to be statistically significant difference, and (P value 0.01 and less) deliberate a highly significant difference.

RESULTS

Table (3-1) shows the mean and standard deviation (±SD) for serum ALP, TP, and UA levels in rabbit's without receiving extract, before and after creating gingival incision wound. From the results, a highly significant increase in serum ALP level (112.25±8.17) has been found, three hours after gingival wound incision. Then this increased value declined step by step at the next time intervals, but still significantly higher than its baseline value before incision. Regarding serum TP in rabbits received distilled water; a significant increase in its level has been observed three hours after oral incision (6.10±0.53), then reaching maximum value after one day of wound incision (7.8±0.398), finally it declined at the other time intervals but still significantly higher than the base line value. A significant increase in serum uric acid in rabbits receiving distilled water was also found, starting from three hours after oral incision, reaching maximum at time interval one day after wound incision (0.35±0.061), finally decline at the other time intervals but still significantly higher than its value before incision.

Table (3-2) shows the mean and standard deviation (±SD) of serum ALP, TP, and uric acid in rabbits receiving pomegranate seed extract, before and after time intervals of creating incision wound. Serum ALP levels showed significant increase, three hours (97.97±8.53) and one day(68.50±5.20), then the degree declined, finally, at the time interval three days and seven days after suture removal, the level returned non- significantly to its baseline value before incision. Regarding serum TP

and serum uric acid, their levels increased significantly, three hours(7.17±0.061 and 0.28±0.11 respectively) and one day(6.90±0.136 and 0.24±0.07 respectively) after oral incision, while at next time interval (three days after incision), their levels returned non- significantly to those before incision.

Table (3-3) shows the comparison of mean value between study group and control group with time intervals after wound incision in gingival rabbit. A highly significant difference in ALP levels was observed, after 3 days and 7 days and a statistically significant difference in TP levels was also seen after 3 days and 7 days. Regarding serum UA levels, both groups didn't show any significant differences with time intervals after wound incision.

Table 3.1: The mean, standard deviation (±SD), and t-test for comparison of serum ALP, TP and UA levels in rabbits, before with each time interval after gingival incision wound receiving water (control group).

Parameters	Time intervals		Mean ± SD	SE	*t*-test	*P*-value
Alkaline phosphates U/L	before incision	baseline	56.97±1.88	1.083		
	after incision	3H	112.25±8.17	4.71	14.71	HS
		1D	97.78±11.61	6.70	6.91	S
		3D	90.27±8.35	4.82	5.91	S
		7D	75.25±5.72	3.31	4.53	S
Total protein (g/dL)	before incision	baseline	5.47±0.67	0.384		
	after incision	3H	6.10±0.53	0.305	7.181	S
		1D	7.8±0.398	0.23	4.689	S
		3D	7.37±0.357	0.206	4.325	S
		7D	6.50±0.15	0.086	4.485	S
Uric acid (mg/dL)	before incision	baseline	0.17±0.115	0.066		
	after incision	3H	0.28±0.104	0.060	7.00	S
		1D	0.35±0.061	0.035	5.29	S
		3D	0.31±0.083	0.048	6.06	S
		7D	0.25±0.10	0.057	6.928	S

Table 3.2: The mean, standard deviation (±SD) and t-test for comparison of serum ALP, TP and UA levels in rabbits, before with each time interval after creating incision wound in their gingiva receiving aqueous pomegranate extract (study group).

Parameters	Time intervals		Mean ± SD	SE	*t*-test	*P*-value
Alkaline phosphates U/L	before incision	baseline	56.97±1.88	1.083		
	after incision	3H	97.97±8.53	4.93	6.856	S
		1D	68.50±5.20	3.00	5.88	S
		3D	60.42±5.91	3.41	1.46	NS
		7D	56.18±3.88	2.24	0.243	NS
Total protein (g/dL)	before incision	baseline	5.47±0.67	0.384		
	after incision	3H	7.17±0.061	0.035	4.328	S
		1D	6.90±0.136	0.078	4.681	S
		3D	5.58±0.23	0.132	0.234	NS
		7D	5.46±0.24	0.138	0.019	NS
Uric acid (mg/dL)	before incision	baseline	0.17±0.115	0.066		
	after incision	3H	0.28±0.11	0.065	5.284	S
		1D	0.24±0.07	0.040	4.40	S
		3D	0.18±0.10	0.060	0.121	NS
		7D	0.16±0.069	0.040	0.062	NS

Table3.3: The comparison between mean values of study group and control group regarding serum ALP, TP and UA levels in rabbits with each time interval after creating incision wound in their gingiva.

Parameters/ Time interval		Study group mean ±SD	Control group mean ±SD	*P*-value
Alkaline phosphates (U/L)	3H	97.97±8.53	112.25±8.17	NS
	1D	68.50±5.20	97.78±11.61	NS
	3D	60.42±5.91	90.27±8.35	S
	7D	56.18±3.88	75.25±5.72	HS
Total protein (g/dL)	3H	7.17±0.061	0.305	NS
	1D	6.90±0.136	0.23	NS
	3D	5.58±0.23	0.206	S
	7D	5.46±0.24	0.086	S
Uric acid (mg/dL)	3H	0.28±0.11	0.060	NS
	1D	0.24±0.07	0.035	NS
	3D	0.18±0.10	0.048	NS
	7D	0.16±0.069	0.057	NS

DISCUSSION

The results of this study showed a higher increase in serum ALP in rabbits receiving distilled water, 3 hours after oral incisional wound, comparing to those receiving pomegranate seed extract. While the value decrease, three days after incisional injury, afterward, the degree still higher than baseline in rabbits receiving distilled water. This result provided evidence that pomegranate has anti-inflammatory and wound healing effects, this is due to that, pomegranate contents particular components called polyphones; that have potential antioxidant, anti-inflammatory, and anti-carcinogenic effects [18]. Alkaline phosphatase (ALP) is a ubiquitous enzyme produced by different cell types. This enzyme has been used as a marker for inflammation and monitoring its response to treatment, since the enzyme levels have been increased in wounds [19, 20]. Using histochemical techniques [21], evaluated the activity of ALP in incisional and excisional acute wounds, chronic wounds, and keloids in animal and human models. Their results showed an increase in ALP level. They concluded that ALP activity is related to acute inflammation and collagen turnover during acute and chronic wound healing. The results of the present study show that total serum protein increased significantly in rabbits receiving distilled water, 3 hours after oral incisional wound, comparing to those receiving pomegranate seed extract. While the value of the study group returned to baseline level, after three days of the incisional cut, in the control group, the level still higher than baseline even seven days after incisional wound. The elevation of serum TP in case of incisional wound indicates the increase in free-radical generation potential, and resulting lipid peroxidation [22]. In case of rabbits receiving pomegranate seed extract, serum TP returned to its baseline value rapidly (after three days of an incisional wound), this indicates the anti-inflammatory, antioxidant, and wound healing effects of pomegranate seed extract which contains a high amount of polyphenol compounds that exerting these effects. The variation between reactive oxygen species production and antioxidant defense determines the degree of oxidative stress [23]. Antioxidants protect the cells from toxins such as free radicals. In addition to antioxidant, Total serum protein, which is a measure of the total amount of proteins in the blood, is considered to be important in the maintenance of health. Antioxidant and Total protein levels were showing general tendency to change during aging [24, 22]. Regarding serum uric acid, the results showed a consequence increase in its level in rabbits receiving distilled water, 3 hours after oral incisional wound, same to those receiving pomegranate seed extract. While the value in the study group returned to baseline level, after 3 days of incisional cut, in the control group, the level still higher than baseline even 7 days after incisional wound. The UA is a potent scavenger of free radicals that provides 60% of free-radical scavenging capacity in plasma [25]. It is regarded as one of the most important antioxidants in the blood of humans [26, 27]. It has been published that serum uric acid elevation was related to the presence of free radical and oxidative stress and higher serum UA concentration was responsible for the elevated serum antioxidant capacity [28]. It has been reported that serum UA levels have been positively associated with both derivatives of reactive oxygen metabolites and biological antioxidant potential levels [29]. It has been found that there was a clear and significant association between UA and several inflammatory markers [30].

Therefore, in the current study uric acid increased, 3hours after oral incisional wound, while its level returned rapidly in case of rabbits receiving pomegranate seed extract. Afore mentioned due to the antioxidant and anti-inflammatory effects of pomegranate that enhanced wound healing. It was displayed that elevated serum UA levels may protect against cancer mortality in a comprehensive study of 1823 males with lung, colorectal and prostate cancer[31]. Therefore uric acid seems to play a dual role in oxidative stress: antioxidant in the extracellular space and pro-oxidant within the cell [32]. The results of circulating UA is a serious antioxidant and might help protect against free-radical oxidative damage [25]. It has been reviewed that there were current evidence regarding the antioxidant role of uric acid and suggested that it has an influential role as an oxidative stress marker and a potential therapeutic role as an antioxidant[28].The increase of serum UA after tissue damage has been shown in a mice model of kidney injury induced by ischemia [33]. They found that, after an ischemic period of 30 min, systemic UA concentration has been significantly elevated but restored to normal within one hour, indicating that elevating serum UA was rapidly reversible.

CONCLUSIONS

From the current investigation, conclusion has been drawn that oral Supplementation of pomegranate seed extract to rabbits can expedite the rate of healing of the oral incisional wound, this effect is due to its content of polyphenol compounds that have free radical scavenging and anti-inflammatory effects.

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المستخلص:

خلفية و اهداف البحث: الرمان ، هي فاكهة صالحة للأكلومستهلكة في جميع أنحاء العالم. الاجزاء الصالحة للأكل من الرمان غنية بمركبات مضاده للأكسدة و مضادة للالتهاب. هدف الدراسة هو التحقيق من تاثيرشراب مستخلص حبات الرمان كمضاد للاكسده ومضاد للالتهاب في تسريع شفاء جروح اللثة عند الارانب .

طرائق البحث: استخدم خمسه واربعون ارنبا ذكرا في الدراسة, وتم تقسيمهم الى ثلاث مجاميع : المجموعة الاولى (مجموعة الاساس)، تتكون من (5) ارانب ،تم اجراء جرح اللثه الشدقي لهم، اما المجموعة الثانية (مجموعة الدراسة) تتالف من (20) ارنباجريت لهم جرح اللثه الشدقي وتعالجوا بمستخلص الايثاني لحبات الرمان. اما المجموعة الثالثة (مجموعه السيطرة) تتالف من (20) ارنبا,اجريت لهم جرح اللثه الشدقي وتركوا بدون علاج, و تمت خياطه الجروح اللثوية الشدقية على القواطع الوسطية اليمنى السفلية، وازيلت الخيوط بعد (7) أيام. جمعت نماذج الدم عن طريق سحبها من القلب خلال فترات زمنية (0 ساعه، 3ساعه، 1 يوم، 3 يوم و 7 أيام) بعد اجراء الجرح.

النتائج: أظهرت النتائج المستحصلة من الدراسة ان هناك زيادة معنوية كبيرة في مستويات نشاط الانزيم الفوسفاتيز القاعدي (ALP) والبروتين الكلي (TP) وحمض البوليك (UA)) في مصل الدم لجميع الفترات الزمنية بعد الجرح اللثوي الشدقي لأرانب مجموعة السيطرة ، في حين ارتفعت مستويات هذه الدالات الكيميائية الحيوية فقط في فترات زمنية معينه: 3 ساعات و 1 يوم بعد الجرح اللثوي الشدقي عند ارانب مجموعة الدراسة (الارانب المعالجة بمستخلص الايثاني لحبات الرمان). اظهرت هذه النتائج بان التغذيه بحبات الرمان له تاثير كمضاد للالتهاب ومضاد للاكسده وبالتالي تسرع من التام جروح اللثه .

الاستنتاجات:ان استخدام حبات الرمان كمكمل غذائي يمكن ان يفعل معدل شفاء جروح اللثه من خلال تاثيره كمضاد للالتهاب وكمضاد للاكسده.