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Serum Muscle Enzymes as Indicator for Gingival Wound Healing in Rabbits Orally Receiving Pomegranate Peel Extract

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ABSTRACT

Background and Objectives: Pomegranate, Punica granatum, is known to possess a powerful ability for accelerating wound healing. The aim of this study is to evaluate serum muscle enzymes; Creatine phospho kinase (CPK), aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) activities in gingival wound healing process in rabbits orally administrated pomegranate peel extract (PPE).

Methods: This study included 55 rabbits that were divided into 3 groups; baseline group consisted of 5 rabbits without creating buccal gingival injury, and the study group included 25 (5 rabbits per each time interval) with creation of gingival injury that received (PPE). The third group; the control group (25 rabbits) was treated as the study group, except they didn't receive the extract. At the lower right central incisor, a gingival injury was created, seven days after the operation the sutured was removed. At time intervals (0, 3h, 1, 3, 7 and 10 days) after the operation, blood was withdrawn through cardiocentesis. Blood samples were used for serum CPK, AST, ALT, LDH activities determination.

Results: In the rabbits receiving PPE (study group), the activities of muscle enzymes; AST, ALT, LDH increased significantly at time intervals of one and three days after gingival injury (except CPK which increased 3 hours, 1 and 3 days after surgery), then declined and nearly returned back to baseline values, 10 days after gingival injury incision. Whereas, in the control group, a significant increase in the serum activities of CPK and AST (compared to baseline values) were observed at all time intervals after gingival injury, while for ALT and LDH, their activities significantly increased one day and after.

Conclusions: Serum muscle enzymes activities may be used as a marker for the gingival wound healing process in rabbits supplemented with PPE.

Keywords: Punica granatum peel extract, Gingival injury healing, Muscle enzymes.

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INTRODUCTION

The healing of wound tissue is a physiological process that repairs tissue injuries and damages. Wound healing occurs in three stages; inflammation, proliferation and migration of different cell types.[1, 2] Free radicals have a negative effect on wound healing processes.[3] Wound healing may be delayed due to a protracted inflammatory period, deferred proliferation and remodeling stages.[4] Many researchers have conducted studies with the aim of accelerating surgical wound healing.[5, 6] Many fruits have the potency to induce and accelerate the wound healing process; these plants have anti-inflammatory, antimicrobial, and antioxidant activities.[6] Pomegranate is such a fruit that has the ability to scavenge free radicals[7] and increases plasma antioxidant capacity.[8] Pomegranate extracts were found to have wound healing properties. However, the ability effects of pomegranate peel extract on surgical injury healing have been poorly studied.[9]

The levels of some serum biochemical contents can be used as indicators or markers for many events, for example, Alkaline Phosphatase (ALP) can be used as a marker for inflammation, tissue damage and the follow up of the healing process.[10,13] Perinetti et al published that ALP activity in gingival crevicular fluid (GCF) can indicate the periodontal healing/recurrent inflammation phases in chronic periodontitis.[14]

Intense prolonged exercise and mechanical or surgical injury can damage muscle tissue.[15,17] Muscle enzymes such as aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), and aldolase can be used in diagnosis of muscle diseases.[18]

Previous studies revealed that serum AST, ALT, LDH, CK, and aldolase can be considered as markers for muscle cell injury, and an increase in these enzymes in serum may be an indicator of muscle stress due to muscle injury or exercise.[19,21] In diverse species of animal, AST, ALT and LDH were found in multiple tissues, including muscle, kidney and liver, these enzymes have been published to be possible markers of heart and muscle injury in humans.[22,23] Monitoring the activities of serum CK, AST, and ALT can help determine if the damaged muscles are recovering as they should be. Elevations in the activity of any of these enzymes for prolonged period of time indicates that the training load is high and requires more time for recovery, thus the overtraining risk may be greater.[24]

Deepika.et al observed an increase in salivary CK,AST and ALP activities in periodontitis patients. They concluded that, when an organ such as the skeletal muscle, heart muscle or periodontium is damaged, the above enzymes will be released into the blood stream or saliva.[25] Todorovic et al, found that changes in the activities of the enzymes; AST, ALT, CK, LDH, ALP, ACP and GGT) reflect metabolic changes in the periodontium and gingiva in inflammation. Their activities returned back to normal values after treatment. Based on their results, they revealed that the activity of these enzymes in saliva can be used as biochemical markers for pe-riodontal tissue damage.[26]

To the best of the author knowledge, no study has been conducted to investigate using muscle enzyme levels as an indicator for the rate of oral wound healing effect of pomegranate supplementation. Therefore, this study was designed to investigate using the serum activity of muscle enzymes; CK, AST, ALT, and LDH as a biochemical marker for evaluating the effect of pomegranate peel extract on the rate of gingival wound healing process.

METHODS

Animal groups:

The study was done in the animal house of the College of Medicine in Hawler Medical University from April to September 2018. The work included 55 healthy male Rabbit (weighing 1.0-1.5 Kg) with the age of 6-8 months. Five Rabbits were left without surgical gingival injury and used as baseline (group 1), while 25 rabbits were orally received PPE solution (100 mg/kg/day), starting from 14 days before the surgical gingival injury, and continuing for 10 days after the operation, according to wound healing model.[27] The third group was the control group, which included 25 rabbits they treated as in the study group, but they didn't receive the extract. All the animals were fed on commercial animal food and water.

Surgical gingival injury creation:

This part was performed by a periodontist. Rabbits were anesthetized by injecting ketamine (40 mg/kg) and xylazine (4 mg/kg), [28] then a surgical injury was made through the marginal gingival,

starting from the gingival sulcus, extending toward the tooth surface to the alveolar crest level .[27,29] Wound was created in the labial aspects the tissue of the lower right central incisor teeth and it was extended through the inter-proximal area. A stitch with (4/0) black silk was used to suture the wound margins. The suture was removed seven days after operation.

Preparation of the Extract:

Pomegranate Peel Extract was prepared according to the Rafraf method.[30] 0.5kg of pomegranate peel was obtained from the fresh fruit and dried at 40°C. The peel particles were finely powdered and extracted in 80% ethanol in water, and then filtered by Whitman paper No. (42), finally the filtrate was dried at 50 °C. 100 mg of the fine dry powder was suspended in one ml. of distilled water. This solution was given orally to the rabbits of the study group.[28]

Starting from 14 days before surgical gingival injury, 1ml (50 mg/kg/day) of PPE suspension were orally given to each rabbit in the study group, and the supplementation was continued till 10 days after operation (according to the wound healing model).[31]

Collection of Blood samples:

Each rabbit was anesthetized, and 3 ml of blood were drawn by cardiocentesis. Blood samples were taken before bone defect incision (0 day) and after wound incision in the following time intervals (3h, 1, 3,7 and 10 days). The blood samples were centrifuged for 10 minutes at 3000 rpm. The serum was separated and used for the determination of serum AST,[32, 33] and ALT,[33, 34] CPK,[35,36] and LDH[37,39] activities using specific kits.

Statistical analysis

The data of the results were analyzed by the statistical package for social science (version 22). All data were expressed as the mean and standard deviation ± (SD). A *t*- test was for comparison two means. A P-value of 0.05 or less was considered to be a statistically significant difference, and P-value of 0.01 and less deliberate a highly significant difference.

RESULTS

Table (1) shows the levels of serum CPK, AST, ALT, LDH activities in rabbit's given PPE (study group) before (at baseline) and after surgically gingival injury creation. A significant increase in serum AST, ALT, LDH activities was found on one day (140.22±10.23, 90.78±8.76, 445±21.43 respectively) and three days (106.33±8.58, 84±7.44, 415±19.71 respectively) after gingival injury creation in comparison to baseline values (71.35±6.11, 67.46±7.35, 330±18.65 respectively). Seven days after gingival injury creating, the serum activities of these enzymes decreased and nearly achieved their baseline values after 10 days from the injury. Regarding serum CPK activity, the results indicated that its activity increased significantly 3 hours, 1 and 3 days after surgical injury (276 ±19.2, 490±30.4, 305±21.6 respectively) comparing to the baseline value (215±15.5), then it decreased and nearly return back to its baseline after 10 days from the injury.

Table (2), shows the levels of serum CPK, AST, ALT, LDH activities in rabbits of the control group, before (at baseline) and after time intervals of creating gingival injury. It was observed that serum CPK, AST activities increased significantly, 3 hours after gingival injury**,** while the activities of serum ALT and LDH increased significantly, 1 day after gingival injury. At the other time intervals after gingival injury creation, the activities of all the enzymes decreased but still significantly higher than the baseline.

Table (3), illustrated the comparison of mean activity ±SD values of muscle enzymes; CPK, AST, ALT, LDH in the study group with those in the control group at different time intervals after creating surgical gingival wound (it was assessed by using Paired sample t-test). Significant differences in the activities of the muscle enzymes were found at time intervals; 3, 7, and 10 days after gingival injury creation between the study group and the control group (except for serum ALT activity; the differences were found at time intervals of 7 and 10 days). In these cases the activities of the enzymes in the control group were higher than the study group.

Table 1: Comparison of the mean Activity ± SD of Serum CPK, AST, ALT and LDH enzymes (time intervals after surgical gingival injury) in the study group to baseline data.

Parameters	Time intervals		No. of Rabbits	Mean ± SD	P-Value
Serum CPK (IU/L)	Baseline	0H	5	215±15.5	
	After surgical gingival injury	3H	5	276 ±19.2	S
		1D	5	490±30.4	S
		3D	5	305±21.6	S
		7D	5	235±16.1	NS
		10D	5	213±14.7	NS
Serum AST (IU/L)	Baseline	0H	5	71.35±6.11	
	After surgical gingival injury	3H	5	80.45±6.85	NS
		1D	5	140.22±10.23	S
		3D	5	106.33±8.58	S
		7D	5	80.65±7.35	NS
		10D	5	74.4±5.94	NS
Serum ALT (IU/L)	Baseline	0H	5	67.46±7.35	
	After surgical gingival injury	3H	5	71.67±7.44	NS
		1D	5	90.78±8.76	S
		3D	5	84±7.44	S
		7D	5	66.98±6.48	NS
		10D	5	63.22±6.7	NS
Serum LDH (IU/L)	Baseline	0H	5	330±18.65	
	After surgical gingival injury	3H	5	345±19.07	NS
		1D	5	445±21.43	S
		3D	5	415±19.71	S
		7D	5	355±18.28	NS
		10D	5	334±16.11	NS

- **H = Hour , D= Day**

Table 2: Comparison of the mean Activity ± SD of Serum CPK, AST, ALTand LDH enzymes (time intervals after surgical gingival injury) in the control group to baseline data.

Parameters	Time intervals		No. of Rabbits	Mean ± SD	P-Value
Serum CPK (IU/L)	Base line	0H	5	205±15.5	
	After surgical gingival injury	3H	5	398 ±20.9	S
		1D	5	520±33.5	S
		3D	5	375±26.8	S
		7D	5	310±21.7	S
		10D	5	281±20.1	S
Serum AST (IU/L)	Base line	0H	5	71.35±6.11	
	After surgical gingival injury	3H	5	87.45±7.25	S
		1D	5	158.25±12.16	S
		3D	5	128.47±1016	S
		7D	5	110.78±8.86	S
		10D	5	90.89±7.50	S
Serum ALT (IU/L)	Baseline	0H	5	67.46±7.35	
	After surgical gingival injury	3H	5	70.36±6.78	NS
		1D	5	113.78±10.07	S
		3D	5	104.21±8.64	S
		7D	5	94.68±6.78	S
		10D	5	88.42±6.73	S
Serum LDH (IU/L)	Baseline	0H	5	322±18.40	
	After surgical gingival injury	3H	5	338± 19.31	NS
		1D	5	475± 23.14	S
		3D	5	467± 20.88	S
		7D	5	415±21.28	S
		10D	5	378±20.13	S

- **H = Hour , D= Day**

Table 3: comparison of the mean activity ±SD of Serum CPK, AST, ALTand LDH enzymes (time intervals after surgical gingival injury) between the study group and the control group.

Parameters	Time interval (Hour;H,Day;D)	Study group (mean ±SD)	Control group (mean ±SD)	P-value
Serum CPK (IU/L)	3H	276 ±19.2	298 ±20.9	NS
	1D	490±30.4	520±33.5	NS
	3D	305±21.6	375±26.8	S
	7D	235±16.1	310±21.7	S
	10D	213±14.7	281±20.1	S
Serum AST (IU/L)	3H	80.45±6.85	87.45±7.25	NS
	1D	140.22±10.23	158.25±12.16	NS
	3D	106.33±8.58	128.47±1016	S
	7D	80.65±7.35	110.78±8.86	S
	10D	74.4±5.94	90.89±7.50	S
Serum ALT (IU/L)	3H	71.67±7.44	70.36±6.78	NS
	1D	90.78±8.76	113.78±10.07	NS
	3D	84±7.44	104.21±8.64	NS
	7D	66.98±6.48	94.68±6.78	S
	10D	63.22±6.7	88.42±6.73	S
Serum LDH (IU/L)	3H	345±19.07	338± 19.31	NS
	1D	445±21.43	475± 23.14	NS
	3D	415±19.71	467± 20.88	S
	7D	355±18.28	415±21.28	S
	10D	334±16.11	378±20.13	S

- **H = Hour , D= Day**

DISCUSSION:

Wound healing process includes inflammation (formation of inflammatory cells), proliferation (epithelialization, angiogenesis and collagen formation) and remodeling (development of fibroblasts, collagen, edema, and blood vessels) phases .[40]The purpose of this study was to investigate the effects of supplementation of PPE on serum muscle enzymes in gingival surgical injury, to evaluate the role of PEE in wound healing process and using serum muscle enzymes as indicator for the rate of gingival wound healing process by PPE in rabbits. The results of the study showed that, all the serum muscle enzymes activities in rabbits supplemented on PPE, increased significantly after surgical gingival injury, but their activities returned back to the baseline values nearly after 7 days, while in control rabbits their activities elevated after surgical gingival injury and still significantly higher after 7 and 10 days. Since serum muscle enzymes act as indicators for muscle injuries and damages and their values return back to the normal range of healing, [41] therefore the results of the serum muscle enzymes activities in the present study indicated that PPE supplementation enhanced and sped up

the gingival wound healing process. Serum activity of muscle enzymes is a potential marker for the muscle function status and can change in both pathological and physiological conditions. Serum CK is believed to be an important indicator for muscle membrane integrity.[42] De Souzaa et al observed that pocket depth and bleeding on probing were associated with changes in CK during training.[43] Alanine Aminotransferase (ALT) activities are elevated with inflammation and periodontitis; which is a chronic inflammation of periodontium. Researchers indicated that such an association exists.[44, 45] A significant increase in the activity of AST was observed in gingival fibroblasts and periodontal ligament fibroblasts, thus AST activity can be used as indicator for the clinical assessment of periodontal disease sites, since AST activity will decrease when periodontal status improves.[46] Many studies have found higher levels of AST in the gingival crevicular fluid (GCF) of the diseased sites than in healthy sites, and have been reported as a possible marker of periodontal active sites.[47,48] It was observed that salivary enzymes such as LDL and Amino transaminases can be used as suitable markers of periodontal disease.[49] Ali et al, reported that the activity of salivary lactate dehydrogenase can be used as biomarker for periodontitis in smokers.[50] Based on their results, Todorovic et al, have assumed that activity of the enzymes; CK, LDH, AST, ALT could be used as biochemical markers for periodontal tissue damage, and might be useful in diagnosis, prognosis and evaluation of therapy effects in periodontal disease.[51] Researchers on rats have found that applying PPE gel topically had improved wound healing process.[52,55]It was published that PP contains phenolic compounds; such as tannins and flavonoids, which can increase fibroblast proliferation and collagen synthesis.[55] KAUR et al , 2006 reported that pomegranate had various pharmacological activities, as well as had the ability of speeding up the process of healing wounds.[56] Studies have shown that Pomegranate parts have antioxidant effects and enhance the activities of enzymes that take part in controlling free radicals, thus may increase the rate of one or more steps in wound healing process.[54,56]

CONCLUSIONS

According to the findings of this study, serum muscle enzymes such as CPK, ALT, AST and LDL can be used successfully as indicators for evaluating the rate of gingival wound healing in rabbits by supplementation of PPE.

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