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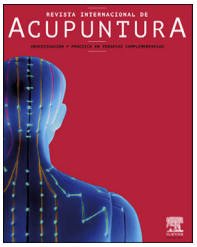

ORIGINAL ARTICLE

The protective effect of both platelet rich plasma and electro-acupuncture on acute pancreatitis caused by tetracycline in rat's model

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KEYWORDS
Electro-acupuncture;
Platelet-rich plasma;
Pancreatitis;
Tetracycline;
Tissue injury

Abstract
Background and objectives: Acute pancreatitis causes about 230,000 of the world's hospitalizations yearly, with mortality rates for severe cases reaching 30%. Drugs are responsible for a large percentage of acute pancreatitis incidents, Based on these health risks, this study investigated the role of platelet-rich plasma and electro-acupuncture as protective therapies against tetracycline pancreatic injury effects.
Materials and methods: This study was carried out on 60 rats after they were divided into 4 subgroups (one control and three treatment groups).
Results: The histological and biochemical examination of the pancreas were shown a promising result about the protective effects of the platelet-rich plasma therapies in which the growth factors may result in the induction of pancreatic cell regeneration and decreased inflammation Also, it found that electro-acupuncture is a promising type of therapy to protect the pancreas from tetracycline toxic effects.
Conclusion: The present study's positive outcomes are hoped to contribute to a decline in the tremendous morbidity and mortality rates from drug-induced pancreatic injury around the world.

PALABRAS CLAVE
Electroacupuntura;
Plasma rico en plaquetas;
Pancreatitis;

Efecto protector del plasma rico en plaquetas y la electroacupuntura en la pancreatitis aguda causada por tetraciclina en un modelo de ratas

Resumen
Antecedentes y objetivos: La pancreatitis aguda causa cerca de 230.000 hospitalizaciones anuales a nivel mundial, con una tasa de mortalidad para los casos graves que roza el 30%. Los

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Tetraciclina;
Lesión tisular

fármacos son responsables de un gran porcentaje de los episodios de pancreatitis aguda. Sobre la base de estos riesgos sanitarios, el presente estudio investigó el papel del plasma rico en plaquetas y la electroacupuntura como terapias protectoras frente a los efectos de las lesiones pancreáticas de la tetraciclina.
Materiales y métodos: Este estudio fue realizado en 60 ratas que fueron divididas en cuatro subgrupos (un grupo control y tres grupos de tratamiento).
Resultados: El examen histológico y bioquímico del páncreas mostraron resultados prometedores acerca de los efectos protectores de las terapias con plasma rico en plaquetas, en los que los factores de crecimiento pueden originar la inducción de regeneración de células pancreáticas y la reducción de la inflamación. De igual modo, se encontró que la electroacupuntura es un tipo de terapia prometedor para proteger el páncreas de los efectos tóxicos de la tetraciclina.
Conclusión: Se espera que los resultados positivos del presente estudio contribuyan a reducir las grandes tasas de morbilidad y mortalidad de las lesiones pancreáticas inducidas por fármacos a nivel mundial.

Introduction

Tetracycline is one of the tetracyclines family of antibiotics that inhibit the synthesis of the protein through the prevention of any attachments between the ribosomal acceptor (A) site and the aminoacyl-tRNA. It is a broad-spectrum agent, exhibiting activity towards a wide variety of the gram-negative (G-) and gram-positive (G+) bacteria, atypical organisms like chlamydia, mycoplasmas, rickettsia, and protozoan parasites.[1]

Acute pancreatitis (AP) is a disorder characterized by acute necro-inflammatory tissue changes that is AP is the first gastrointestinal disorder that require admission and the 21th among all diseases that call for hospitalizations with estimated costs of approximately 2.6 billion dollars per year,[2] the urgent need for the AP prevention techniques comes from the fact that it is a potentially fatal disease that has high morbidity and significant mortality (2.1%–7.8) and as shown in several studies[3] AP following Tetracycline administration has been related to the fatty degeneration and acinar cell destruction that can be identified histologically and biochemically by rising serum pancreatic enzymes.[4] Tetracycline also causes small but considerable oxidative stress in the pancreas, where the levels of the free radical have exhibited a considerable increase. This is clearly shown when antioxidant enzymes like catalase, glutathione reductase, glutathione peroxidase, and superoxide dismutase have shown a small but significant decrease in the treated rats' pancreas at a dose of 50mg/kg/day.[5]

Platelet Rich Plasma (PRP) can be described as an autologous concentration of human platelet to the supra-physiologic levels.[6] At the baseline levels, the platelets act as a natural reservoir for the growth factors (GFs) including the epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-β1), hepatocyte growth factor (HGF), basic fibroblast growth factor (FGF), and insulin-like growth factor (IGF-I),[7] For example, those GFs are released from activated platelets' alpha granules and have an impact on significant cellular processes, for example chemotaxis, mitogenesis, metabolism, and differentiation.[8] This is why the increase of the platelets' concentration in the injured (i.e., compromised) tissues lies in the belief that the additional platelets result in an exponential release of multiple bio-active factors and, as a result, improve the natural process of healing. The PRP is produced from the blood in a way that guarantees a high platelet concentration in a small plasma volume. After that, this platelet-rich product will be re-injected into the injury site or produced as gel or another bio-material to be inserted during surgery.[9]

Electro-acupuncture is an old medical treatment that originated in China approximately 20 centuries ago. Following the long-term developments, electro-acupuncture has been included in the medical literature as one of the standard therapies in China and presented to other regions and countries like Europe, Asia, and the U.S.A.[10] Electro-acupuncture has shown its importance in promoting gastrointestinal function and improving intestinal paralysis in AP patients. Several studies have shown that electro-acupuncture is effective in gastrointestinal physiology, such as gastric acid secretion and neurohormonal changes. This is why it may result in improving gastrointestinal function in patients with acute pancreatitis.[11] A clinical trial has shown that electro-acupuncture may result in reducing AP severity by induction of the anti-inflammatory effects.[12]

Nonetheless, evidence for electro-acupuncture as one of the adjuvant treatments for the AP is still not credible owing to the lack of well-performed clinical studies. So this research is aimed at assessing the safety and efficacy of electro-acupuncture as one of the adjuvant treatments for acute pancreatitis.

Aim of the study

The objective of the present study is to find the protective effect of PRP and electro-acupuncture on AP caused by tetracycline, as this disease results in several hospitalization

cases worldwide with mortality rates for severe cases that may reach up to 30%, and For treating Acute Pancreatitis. There are a few high-quality clinical researches on electro-acupuncture applications and PRP available concering this subject.[13]

Materials and methods

Animals

The present study has been carried out during January 2021 and conducted according to the ethical principles of the national and international research ethics committee that are reviewed and certified by karbala university, college of pharmacy. All protocols were done according to "Guide for the Care and Use of Laboratory Animals" (NRCotN, 2011) (Ethics Code:IR.QUMS.REC.1399.269).

An average of 60 pathogenically free adult male rats were involved in the study, and their weight was about 150–200 gm, and they were 8–10 weeks old. The animals have been obtained from the pharmacology department's animal house in the college. Rats have been housed in clean plastic cages and were administered a standard lab diet with free access to the water and diet at room temperature with the normal dark and light cycles. All aspects of the animal treatment and care have been performed based on the local rules of CPCSEA (i.e., the Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental design

The 60 rats were divided into 4 equal groups (14 rats each) as shown in table 1 and the remaining 4 rats were used for PRP preparation.

Reagents and therapy

Tetracycline dose preparation

A Tetracycline capsule (250 mg) was used to prepare each dose by direct powder dissolving in normal saline. Each rat in the treatment groups[2–4] received tetracycline at a dose of 50mg/kg (the dose standardized according to the local experiment circumstances) once daily for 21 days by intraperitoneal injection (IP) for induction of pancreatic injury.[5]

PRP method

PRP preparation was performed through the adaptation of the protocol of a double centrifugation tube approach[14] briefly, 4 rats have been anesthetized with the use of xylazine and ketamine, 0.5 ml of blood has been obtained from each rat under aseptic conditions from heart and collected into EDTA tubes for their anticoagulant effect. Blood has been subjected to the approach of double centrifugation method; in the 1st step of centrifugation, the tube has been centrifuged at a 1500 rpm for 5 min, and this step gives rise to three layers; the lower layer included red blood cells, the intermediate one included buffy coat of white blood cells, and the higher layer contained plasma. The plasma layer has been separated, then centrifuged again at 3500 rpm for 5 minutes, then transferred to a vortex for 2 minutes, and the lower part was taken, representing the PRP. Each rat in group 3 received 0.1 ml of the PRP by subcutaneous (SC) injection using a sterile insulin syringe (one dose) once through the first week of the experiment as the PRP may need up to 2 weeks to exert its full effect.[15]

Table 1 Experimental design.

NO.	Group	Characteristic
1	Control	didn't receive any reagents or therapy.
2	Tetracycline	Received only tetracycline.
3	Tetracycline + PRP	Received both Tetracycline and PRP.
4	Tetracycline + Electro-electro-acupuncture	Received tetracycline and electro-electro-acupuncture.

Electro-acupuncture method

In the current study, the electroacupuncture device used is the "Agilent B1500A semiconductor analyzer" to generate electrical pulses to stimulate targeted acupuncture points (Sp6). The pulse includes a continuous square wave with a voltage range of 0 to 0.5, at 8Hz. And all subjects treated for one minute, in a range of three times weekly for 21 days. The 4th group of rats received tetracycline plus electro-acupuncture.

Statistical analysis

The stratistical analysis done using statistical packages of social scinces (SPSS vr. 28)

Histological study

Besides controls, treated rats were sacrificed on day 22 of the experiment after they were anesthetized by chloroform, a method approved by CPCSEA. The histological preparations of the samples included four steps : fixing, processing, sectioning, and staining. The pancreatic specimens were fixed in 10% buffered formalin for 24 hours (a formalin-to-specimen ratio of 10:1 was used for best results) to preserve the most natural possible states and to prevent decay. Then the samples are dehydrated in graded alcohol series to remove any water and cleared in xylene. The embedding step is done by means of an embedding center. Samples are sited in a mold filled with molten paraffin and are carefully oriented according to their plane of section. The resulting block is then cooled. Sections of 5 have been mounted on the glass slides using microtomy and finally the staining was made with H&E (i.e., hematoxylin and eosin) and other sections with Masson trichrome to provide contrast to tissue sections and make the sample structures viewable easier according to histological preparation standards.

Biochemical study

Serum activitiy of amylase and lipase was determined on day 22 of the experiment for each rat via laboratory kit according to the manufacturer guidelines of the Roche-Hitachi modular analytics system (Roche, Mannheim, Germany).

Results

Histological analysis

The 1st group (control) is seen with normal pancreatic tissue formed by exocrine and endocrine (islands of Langerhans) as shown in Fig. 1a. In contrast, in the 2nd group, which received tetracycline drugs, the pancreatic tissue was found with severe acute inflammatory cells in peripancreatic tissue in addition to strongly inflamed acini and fat necrosis as shown in Fig. 1b. In the 3rd group, which was given Tetracycline plus PRP, the pancreatic tissue showed a moderate number of inflammatory cells in the stroma (interlobular connective tissue) in comparison to the 2nd group (tetracycline) in addition to the decreased intensity of inflammation in peripancreatic tissue (fatty tissue) as seen in Fig. 1c. Finally, in the 4th group, which was treated with Tetracycline and electro-acupuncture, the pancreatic tissue was seen with low grade pancreatitis (interlobular connective tissue) and minor peripancreatic inflammation, and most of the tissues appeared in normal condition., as shown in Fig. 1d.

Biochemical analysis

The data showed a significant difference in both amylase and lipase concentration in the experimental groups where they were both within the normal value in the control group and within a very high value in the tetracycline receiving rat group, reflecting pancreatitis and organ damage, whereas both enzyme levels shifted toward the normal values in the third and fourth groups as an index of the protective effect of both the PRP and the electro-acupuncture against the toxic effect of tetracycline on the pancreas and as shown in Table 2.

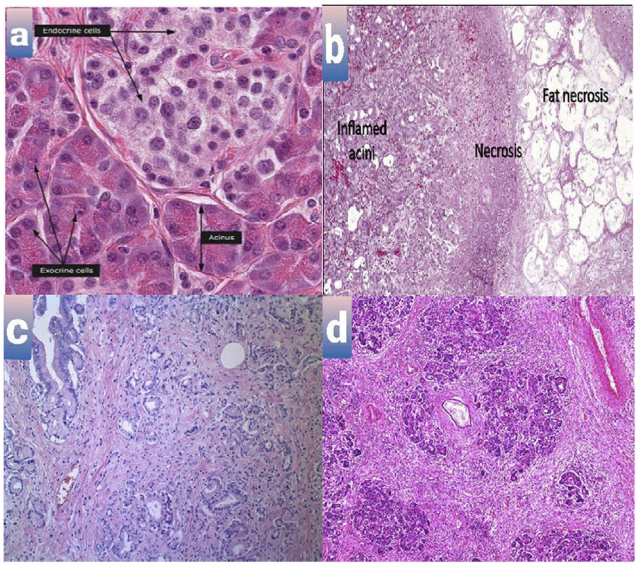

Fig. 1 Histopathogical features of a. Control group, b. Tetracycline group, c.Tetracycline+PRP group, d. Tetracycline + acupuncture group. Magnification is 400x.

Discussion

The pathophysiology of acute pancreatitis includes the activation and release of pancreatic enzymes in the plasma, the destruction and malformation of the pancreatic cells, and organ dysfunction[16] So the present study involved both histological and biochemical analysis to assess the protective effect in a complete manner. Amylase and lipase serum levels rise in the hours following pancreatic damage, and a concentration of 2-4 times the maximum points of normal levels is recommended for pancreatitis diagnosis.[17]

The first significant result of this study is that tetracycline is a causative factor for pancreatic injury in rats at a dose of 50 mg/kg daily for 21 days. This result is supported by many previous studies that confirmed the antibiotic tetracycline as a pancreatitis-causing factor,[18,19] This adverse event may unpredictably occur through the irregular interactions between the organism and the medicine, often mediated by the cytotoxic or immunologic effects that are triggered by the medicine or its metabolites in the pancreas, resulting in the activation of the inflammatory response, which results in increasing the pancreas' vascular permeability. Edema, hemorrhage, necrosis, and ischemia may follow.[20] AP severity may differ while it progresses to a systemic inflammatory response syndrome, sepsis, or multiple organ failure.[21] Approximately 3-13% of AP cases progress to chronic pancreatitis.[4]

In this literature, the promising results are the positive effects of the PRP injections on regenerating the pancreatic cell mass and decreasing inflammation associated with the administrated tetracycline, and this result is consistent with numerous trials that proved the protective effects of the PRP. For example, one of the Egyptian studies showed that the PRP's growth factors could regenerate the pancreatic beta cells in T2DM (Type Two Diabetes Mellitus).[22] Another histological and immuno-histochemical study in 2017 demonstrated the therapeutic effects of the PRP Injection on the Endocrine Pancreas of Experimentally Induced Diabetes in Male Albino Rats.[23] This effect is due to the fact that PRP is 2–5 times richer in platelets than circulating blood,[24] whereas in our work, the preparation of the PRP depends on the double centrifugation approach, which resulted in a concentration of the platelets that is 3 times greater compared to the initial blood sample. Moreover, the rationale for the widespread use of PRP for healing purposes lies in the fact that platelets represent a reservoir of the critical GFs, cytokines, and fibrinogen that can control and regulate the tissue healing process.[25]

Electro-acupuncture therapies can be used along with medical treatment to help the patient feel better,[26] and this type of traditional medicine will pave the way for a novel treatment of organ damage introduced by drugs or any other factor. In the present study, encouraging results about the electro-acupuncture protective effect were gained for further investigation into the exact mechanism, onset and duration of action and the protection degree. Previous animal research has concluded that electro-acupuncture may result in the inhibition of the nuclear factor-kB activation process in the pancreas of rats that have acute pancreatitis and decrease the release of the serum proinflammatory cytokines interleukin 6 (IL6) and tumor necrosis

Table 2 Serum lipase & amylase mean concentrations in the experimental groups.

Parameter	Control	Tetracycline	Tetracycline + PRP	Tetracycline + electro-acupuncture	P value
Serum lipase (IU/L)	34 .1 ± 7 [a]	98 .5 ± 8[b]	63.8 ± 2[ab]	41 ± 6[a]	>0.05
Serum amylase (IU/L)	73.9 ± 5[a]	261.3 ± 4[b]	138.6 ± 3[ab]	100.2 ± 5[a]	>0.05

* Values are expressed as mean ± s.d for 15 rats in each group.
* Small different letters mean significant differences (p value >0.05).

factor (TNF)[27] At the same time, electro-acupuncture may re-constitute a balance between the anti-inflammatory and proinflammatory cytokines. As a result, the pancreatic damage and the systemic inflammatory responses will be reduced.[28] In addition to that, electro-acupuncture may result in a significant reduction in the colonic transmission time, regulation of the gastrointestinal hormone releases, and improvement of the gastrointestinal motility disorders in patients with acute pancreatitis,[29,30] Additionally, other research work[31] Several mechanisms relating to electro-acupuncture must still be investigated based on their gene expression patterns in order to reveal specific details involved in its action. On the other hand, plasma rich platelets contain several growth factors, which play multiple pathways in the human body, including systemic signaling pathways in addition to local effects.[32]

Conclusion

Electro-acupuncture is a promising alternative medicine to protect the pancreas from tetracycline toxic effects and can play a role in tissue regeneration and reformation process in addition, PRP is viewed as a natural biological mediator controlling the growth, differentiation. GFs in the PRP can induce pancreatic cell regeneration and decrease inflammation.

Recommendations

Electro-acupuncture and PRP are recommended as complementary therapies for acute pancreatitis caused by tetracycline.

Conflict of interest

None.

References

1. Rawla P, Sunkara T, Thandra KC. Gaduputi V "Hypertriglyceridemia-induced pancreatitis:". Clin. J. Gastroenterol. December 2018;11(6):441–8.
2. Garber A, Frakes C, Arora Z, Chahal P. Mechanisms and management of acute pancreatitis. Gastroenterol. Res. Pract. 2018 Mar 15:2018.
3. Whalen K. Lippincott illustrated reviews: pharmacology. Lippincott Williams & Wilkins; 2018 Aug 14.
4. Sarojini D, Sunitha P, Kanthal LK, Lavanya K, Bhar Kausik. A study on clinical profile of patients with acute severe pancreatitis on carbapenem versus non-carbapenems. Res. J. Pharmacol. Pharmacodyn. 2012;4(6):349–52.
5. Gangawane Prashant, Sayed Usha. Dyeing of silk with discarded tetracycline hydrochloride drug. Asian J. Pharm. Tech. Jan.-Mar. 2013;3(1):34–6.
6. Vijayalakshmi B, Pradeep S. Role of platelet rich fibrin in dentistry. Res. J. Pharm. and Tech. 2016;9(11):2037–40.
7. Ali Samara M, Mohammed Sarmad MH, Zeiny Mohammed Qasim, AlAtrakji MalAllah. Effect of Prp on gingival health in pregnants: immunological aspect. Res. J. Pharm. and Tech. 2017;10(11): 3969–73.
8. Sowmya MV, Mangayarkarasi M. Effect of physiotherapy intervention after platelet rich plasma procedure in subjects with grade 3 osteoarthritis knee. Res. J. Pharm. and Tech. 2020;13 (5):2065–8.
9. Masoudi EA, Ribas J, Kaushik G, Leijten J, Khademhosseini A. Platelet-rich blood derivatives for stem cell-based tissue engineering and regeneration. Curr. Stem Cell Rep. 2016 Mar 1;2(1):33–42.
10. Zhang K, Gao C, Li C, Li Y, Wang S, Tang Q, Zhao C, Zhai J. Electro-acupuncture for acute pancreatitis: a systematic review and meta-analysis. Pancreas. 2019 Oct 1;48(9):1136–47.
11. Indra V. A study to assess the effectiveness of acupressure during labour pain among primigravida mothers in selected hospitals, Puducherry. Int. J. Adv. Nur. Manag. 2017;5(2):99–104.
12. Li L, Yu J, Mu R, Dong S. Clinical effect of electroelectro-acupuncture on lung injury patients caused by severe acute pancreatitis. Evid. Based Complement. Alternat. Med. 2017 Jan 1:2017.
13. Swathy S, Sethu V Gowri. Electro-acupuncture and lower back pain. Res. J. Pharm. and Tech. August, 2015;8(8):991–3.
14. Xu Q, Chen J, Cheng L. Comparison of platelet rich plasma and corticosteroids in the management of lateral epicondylitis: a meta-analysis of randomized controlled trials. Int. J. Surgery (London, England). July 2019;67:37–46.
15. Almaali HMA, Zghair MA, Farhan NH. The protective effects of platelet rich plasma in diclofenac sodium induced toxic destructive effects in rats' models Lat. Am. J. Pharm. April 2021;40(special issue):1–365.
16. Gupte AR, Forsmark CE. Chronic pancreatitis. Curr. Opin. Gastroenterol. 2014 Sep 1;30(5):500–5.
17. Jagadeesan B, Hari Haran P, Praveen D, Ranadheer Chowdary P, Vijey Aanandhi M. A comprehensive review on pancreatic cancer. Res. J. Pharm. and Tech. 2021;14(1):552–4.
18. Wolfe D, Kanji S, Yazdi F, Barbeau P, Rice D, Beck A, Butler C, Esmaeilisaraji L, Skidmore B, Moher D, Hutton B. Drug induced pancreatitis: a systematic review of case reports to determine potential drug associations. PLoS One. 2020 Apr 17;15(4), e0231883.
19. Ibadi Mohammed Hamzah, Abood Sameer H, Al-Rekabi, Abadi Hassan Abdulla. A study of the effect of octreotide and N-acetylcysteine on inflammatory and prognostic parameters in severe acute pancreatitis in rats. Res. J. Pharm. and Tech. 2018;11(12):5461–8.
20. Kaurich T. Drug-induced acute pancreatitis. Baylor University Medical Center Proceedings. Taylor & Francis; 2008 Jan 1. p. 77–81. No. 1.
21. Bharathi S. Management of chronic pancreatitis: a review. Res. J. Pharm. and Tech. August, 2015;8(8):1083–6.

22. Sarojini D, Sunitha P, Kanthal LK, Lavanya K, Bhar Kausik. A study on clinical profile of patients with acute severe pancreatitis on carbapenem versus non-carbapenems. Res. J. Pharmacol. Pharmacodyn. 2012;4(6):349–52.
23. Zhu X, Yang L, Li X, Zhu F, Li Z, Craemer A, Xiong Y, Lan Y, Zhao Y, Wu J. Efficacy and safety of electro-acupuncture on relieving abdominal pain and distension for acute pancreatitis: a protocol for systematic review. Medicine. 2020 Feb;99(8).
24. Alves R, Grimalt R. A review of platelet-rich plasma: history, biology, mechanism of action, and classification. Skin Append. Dis. 2018;4(1):18–24.
25. Cole BJ, Seroyer ST, Filardo G, Bajaj S, Fortier LA. Platelet-rich plasma: where are we now and where are we going? Sports Health. 2010 May;2(3):203–10.
26. Juel J, Liguori S, Liguori A, Poulsen JL, Valeriani M, Graversen C, Olesen SS, Drewes AM. Electro-acupuncture for pain in chronic pancreatitis: a single-blinded randomized crossover trial. Pancreas. 2017 Feb 1;46(2):170–6.
27. Juel J, Liguori S, Liguori A, Poulsen JL, Valeriani M, Graversen C, Olesen SS, Drewes AM. Electro-acupuncture for pain in chronic pancreatitis: a single-blinded randomized crossover trial. Pancreas. 2017 Feb 1;46(2):170–6.
28. Xue QM, Huang L, Li N. Effects of electro-acupuncture at Tianshu (ST25) on pro-and anti-inflammatory cytokines in rats with severe acute pancreatitis. Zhong xi yi jie he xue bao= Journal of Chinese Integrative Medicine. 2011 Jun;9(6):658.
29. Jang DK, Jung CY, Kim KH, Lee JK. Electro-acupuncture for abdominal pain relief in patients with acute pancreatitis: study protocol for a randomized controlled trial. Trials. 2018 Dec;19 (1):1–6.
30. Swathy S, Sethu V Gowri. Electro-acupuncture and lower back pain. Res. J. Pharm. and Tech. August, 2015;8(8):991–3.
31. Zhang Luyao, Zhiyang Wu, Zhou Jing, Shengfeng Lu, Wang Chaofan, Xia Yiqiu, Ren Hongyan, Zhihui Tong Lu, Ke and Weiqin Li. Electroacupuncture ameliorates acute pancreatitis: a role for the vagus nerve–mediated cholinergic anti-inflammatory pathway. Front. Mol. Biosci. 2021 https://doi.org/10.3389/fmolb.2021.647647 May 13;8:647647.
32. Wasterlain Amy S, Braun Hillary J, Harris Alex HS, Kim Hyeon-Joo, Dragoo Jason L. The systemic effects of platelet-rich plasma injection. Am. J. Sports Med. 2013 Jan;41(1):186–93.