Histological Effect of Platelet Rich Plasma on CCL4 Induced Liver Fibrosis in Adult Albino Rat

Original Article

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ABSTRACT

Background: Liver cirrhosis is an important lethal health problem all over the world. Liver transplantation is the first efficient treatment. Unfortunately, it is limited due to many obstacles. Therefore, alternative treatments are required for cirrhosis. Platelet rich plasma (PRP) is a challenging measure for treatment of cirrhosis.

Aim of the Work: To assess the effect of PRP on experimentally induced liver fibrosis in albino rats.

Material and Methods: Twenty-four adult female rats were included in this study. They were divided into three groups: Group I: a control group and included 6 adult rats that received 0.5 ml of olive oil. Group II: consisted of 12 rats. They were subdivided into subgroup IIA: it included 6 rats that received carbon tetrachloride (CCL4) for six weeks and were sacrificed after the last injection for induction of fibrosis, subgroup IIB: it included 6 rats that were left for spontaneous recovery for four weeks after the last CCL4 injection. Group III: it consisted of 6 rats that were subjected to fibrosis then they received PRP (1 ml\kg) twice weekly for four weeks and then were sacrificed. Liver specimens were prepared for histological and immune-histochemical techniques. Morphometrical and statistical studies were done.

Results: CCL4 injection resulted in multiple injuries in hepatocytes including vacuolations in the cytoplasm and pyknotic nuclei. In addition, it resulted in hepatic fibrosis especially surrounding the central vein and portal areas. Treatment by PRP resulted in an obvious histological improvement in hepatic structure including apparently normal liver cells with acidophilic cytoplasm and vesicular nuclei and significant decrease ($P<0.05$) in hepatic fibrosis. Unfortunately, few areas in the field of hepatic tissue were not improved. There was significant increase in the mean number of Proliferating Cell Nuclear Antigen (PCNA).

Conclusion: PRP could be an adjuvant treatment for hepatic fibrosis which needs more investigations.

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Key Words: CCL4, liver fibrosis, PRP, rat.

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INTRODUCTION

Liver cirrhosis is an important, lethal and major health problem all over the world. It is a severe type of liver fibrosis occurring due to chronic hepatic damage[1]. Liver fibrosis may happen due to many etiologies such as bilharziasis, alcohol intake, obesity, metabolic syndromes, cholestasis, steatosis, viral infections and different hepatic toxins[2]. About 64% of cirrhotic patients suffer from thrombocytopenia[3] and occasionally cannot take antiviral treatment by adequate doses of interferon for viral hepatitis or therapeutic surgery for hepatic carcinoma. Liver transplantation is the only efficient treatment after failure of standard therapy. Unfortunately, liver transplantation is limited by unavailability of donors, post-surgical problems, organs rejection and very expensive[4]. Therefore, alternative treatments are required for treatment of cirrhosis, and PRP is one of the challenging measures for treatment of cirrhosis.

Carbon tetrachloride (CCL4) is a broadly used chemical for induction of fatty liver and liver fibrosis experimentally in animals[1].

The influence of platelet on hepatic proliferation and regeneration was originally recorded by Lesurtel *et al*., in (2006) who recommended that platelet serotonin[5] is very essential for the process of hepatic regeneration. Platelet rich plasma (PRP) provides a developing area for researchers and clinicians. Platelets contain numerous growth factors (GFs). For example, transforming growth factor (TGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and insulin like growth factor (IGF)[6]. Nevertheless, there are few researches on the role of PRP in hepatic regeneration in rats which could not reveal the association between platelets and liver regeneration[7]. Recently, with advances in PRP devices and preparation methods, PRP shows a promising patient outcome results in many diseases and disorders[8].

MATERIAL AND METHODS

Animals

Twenty-four adult female rats aging five to six months, of average weight (200gm) were included in this study.

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In addition, twenty young-weaned albino rats weighing 70-80 gm each, aging about one month, were used for PRP preparation.

The rats were maintained at the Medical Research Centre at Faculty of Medicine, Ain Shams University with free access to water and food. They were housed in plastic cages with mesh wire covers and were kept under suitable circumstances of light, temperature and humidity.

The interventions were performed according to the guidelines of Animal Care of the Scientific Research Ethical Committee of the Faculty of Medicine, Ain Shams University.

Experimental protocol

Rats were maintained for 7 days before starting the experiment for acclimatization. Then they were divided randomly into three main groups:

Group I: Control group: 6 rats received 0.5 ml of olive oil intraperitoneal (vehicle of CCL4).

Group II: CCL4 treated group: 12 rats for induction of liver fibrosis and were subdivided into:

Subgroup IIA: received CCL4 by intraperitoneal injection in dose of 0.5 mg/kg twice weekly[9] for 6 weeks then were sacrificed. (20 mgs of CCL4 were dissolved in 100 mls of olive oil. Each rat will receive 0.5 ml of the solution twice weekly).

Subgroup IIB: received CCL4 in the same dose and route as subgroup IIA but were sacrificed 4 weeks after the last injection.

Group III: treated group: 6 rats received CCL4 for induction of fibrosis as in group II for 6 weeks then received PRP injection subcutaneously in a dose of 1ml/kg 2 days weekly for another 4 weeks and then were sacrificed.

Preparation of platelet rich plasma (PRP)[10]

The blood (2 ml) was taken from the retro-orbital artery of each animal and assembled into sodium citrate containing tubes.

The samples underwent first centrifugation about 8 minutes.

The samples displayed 3 various density compartments, the bottom was (RBCs), the middle was the buffy coat (WBCs), and the top was plasma.

The plasma gave 3 different layers. The first top layer was platelet poor plasma (PPP), the middle was platelet average plasma (PAP) and the bottom was (PRP).

The 1st and the 2nd were eliminated

The 3rd was taken gently and underwent second centrifugation about 5 minutes.

Then the 1st plasma layer was removed, and the 2nd one (PRP) was gathered for use to treat rats with induced hepatic fibrosis.

Platelets were counted by the hematology analyzer device (900,000-1,000,000 cells/μL).

All doses of PRP were freshly prepared and freshly injected into the diseased rats.

Histological study

At the end of experiment, all rats were anesthetized by ether, liver samples were collected. The samples were fixed using10% formalin solution for preparation of paraffin blocks. Paraffin sections of 5 μm thick were obtained and stained with H&E, Masson's Trichrome stain for collagen fibers[11] and PCNA for proliferation assay[12]. The primary antibody used was monoclonal mouse anti-human antibody and the secondary was biotinylated anti mouse antibody.

Morphometric study and Statistical analysis

LEICA Q win Image Analyzer, Histology Department Faculty of Medicine Ain Shams University was used to measure the Mean area percentage of collagen fibers in Masson´s trichrome stained sections (X20) and the mean number of PCNA positive cells (X40). The parameters were measured in five separate non overlapping fields in five sections from each rat of five different rats of all groups. Values were stated as mean ± SD.

Statistical analysis was carried out with the SPSS computer program (version 20) using ANOVA-one-way analysis to compare means of all subgroups. Differences were considered significant when the *P value* was < 0.05.

RESULTS

H & E stained sections

Examination of H & E stained sections of (group I) revealed that the liver appeared to be composed of ill-defined hexagonal classic lobules. Each lobule was traversed centrally by a central vein. The parenchyma of these lobules was composed of liver cells (hepatocytes). The hepatocytes appeared to be organized as branching sheets which radiate from the central vein to the classic hepatic lobules periphery. They were separated by hepatic sinusoids, that were lined with flat endothelial cells. The liver cells showed acidophilic granular cytoplasm with single central rounded open-face nuclei and some of them appeared binucleated (Figure 1a). At the periphery of the classic hepatic lobules, portal areas were noticed. The portal area was composed of branches of hepatic artery, portal vein and bile duct (Figure 1b)

Examination of H and E stained histological sections of the liver of rats that received CCL4 injections for six weeks (subgroup IIA) revealed distorted hepatic architecture in most of the fields, many hepatocytes were vacuolated. They contained multiple, large cytoplasmic vacuoles and densely stained nuclei (Figures 1c,1d). Dilated hepatic blood sinusoids were found (Figure 1c). Congestion of the blood vessels was also found in the blood vessels in the portal areas. Mononuclear inflammatory infiltrate was seen in-between the affected hepatocytes (Figure 1d).

Examination of H and E stained histological sections of rats livers that received CCL4 injections for six weeks and sacrificed after another 4 weeks (subgroup IIB) showed loss of hepatic architecture and presence of regenerative nodules of hepatocytes surrounded by fibrous tissue and inflammatory infiltrate (Figures 1e,1f).

Examination of liver sections of (group III) showed more or less normally appeared hepatic architecture in many fields. Central vein (CV) was surrounded by branched cords of liver cells with granular acidophilic cytoplasm and open-face nuclei and separated with hepatic blood sinusoids that were still congested, few hepatocytes still showed small pyknotic nuclei (Figure 1g). Hepatocytes in most of the fields showed marked decrease in the vacuolations. Moreover, some portal areas appeared with congested blood vessels and surounded with mild inflammatory infiltrate (Figure 1h).

Masson's trichrome-stained sections

Control group showed that the hepatic parenchyma was supported by a stroma of very fine network of collagen. Few collagen fibers were seen encircling the central vein (Figure 2a) and in the portal areas (Figure 2b). The mean area percentage of collagen fibers were (12.1±1.7, Histogram 1).

Subgroup (IIA) exhibited increased amount of collagen fibers deposition surrounding the central vein (Figure 2c) and in the portal area (Figure 2d). The mean area percentage of collagen was significantly increased comparing with the control group (P<0.05), (43.7±6.7, Histogram 1).

Subgroup (IIB) showed more increase in collagen fibers deposition surrounding regenerative liver nodules (Figures 2e,2f). The mean collagen area percentage was measured (82.5± 4.6) which was significantly increased (P<0.05) comparing with both control group and subgroup IIA (Histogram 1).

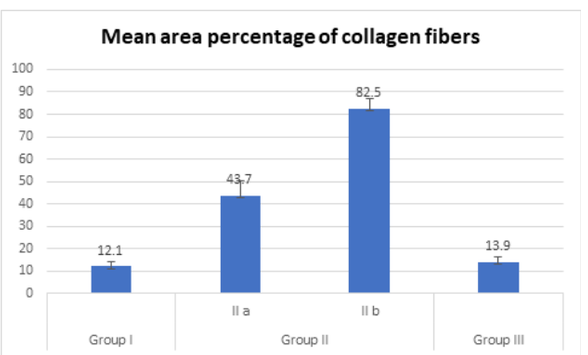
Group III showed few collagen fibers surrounding the central vein (Figure 2h) and in the portal tract (Figure 2i) comparing with group IIA and group IIB. The mean area percentage of collagen was measured (13.9±2.7) which was significantly decreased (P<0.05) comparing with both subgroups IIA and IIB and non-significantly increased (Figure 2g) (P>0.05) comparing with control group (Histogram 1).

C – Proliferating Cell Nuclear Antigen (PCNA) positive cells

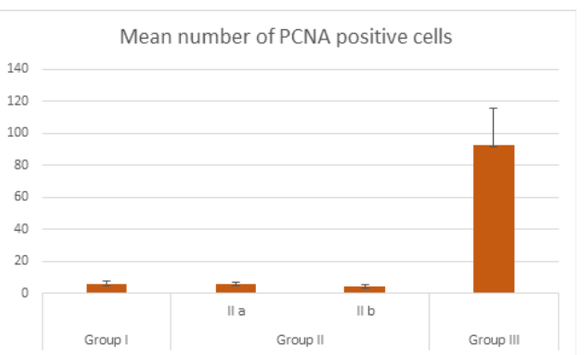
The control group by using streptavidin–biotin immunoperoxidase technique for PCNA, revealed that few liver cells showed positive brown PCNA reaction (Figure 3a). The mean number of PCNA positive cells was measured (6.1±1.5, Histogram 2)

Both subgroups IIA and IIB showed positive brown PCNA reaction in few cells (Figures 3b,3c). The mean number of PCNA positive cells was measured (subgroup IIA 5.7±1.7, subgroup IIB 4.5±1.1) which was non-significant difference as compared to the control group (P> 0.05) (Histogram 2).

Group III showed significant increase in the number of PCNA positive cells (Figure 3d). the mean number of PCNA positive cells was measured (92.8±22.8) which was significantly increased (P< 0.05) as compared to control and subgroups IIA and IIB (Histogram 2).



Histogram 1: showing the mean area percentage of collagen fibers



Histogram 2: showing the mean number of PCNA positive cells

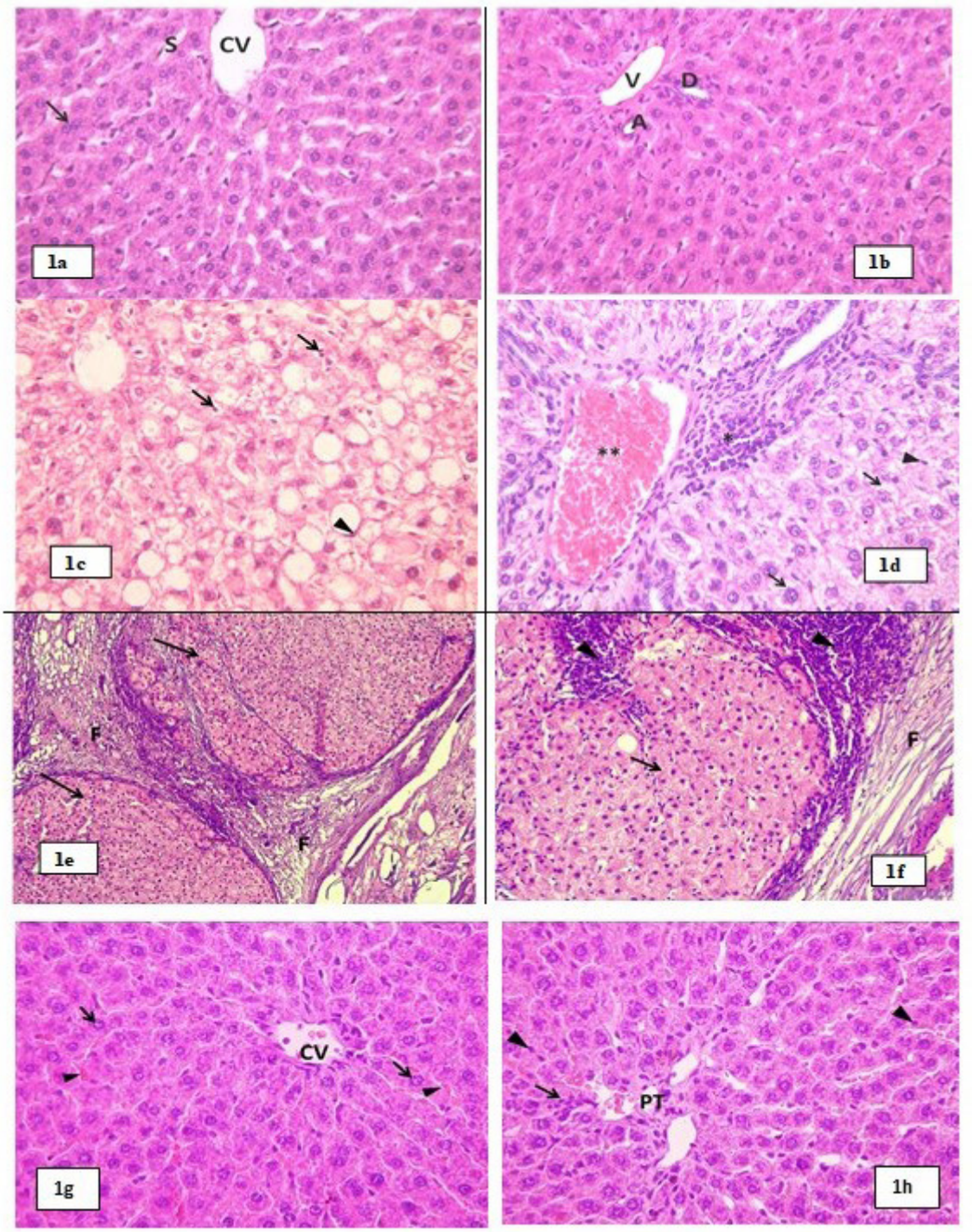


Fig. 1: H&E-stained section: (Fig. 1a): Control group revealed central vein (CV) and cords of liver cells, with central open-face nuclei (↑), blood sinusoids in between (S). Fig. 1b: control group revealed portal area containing branch of portal vein (V), branch of hepatic artery (A) and bile duct (D). Fig. 1c: Subgroup IIA showing dilated hepatic sinusoids (▲) and Some cells appeared with small pyknotic nuclei (↑). Notice, the cells near the sinusoids were less affected. Fig. 1d: supgroup IIA showing congested portal vein branch (**), mononuclear cell infilterate (*), some hepatocytes with vacuolated cytoplasm (↑) and small pyknotic nuclei (▲). Fig. 1e: subgroup IIB showing loss of hepatic architecture and presence of regenerative nodules (↑) of hepatocytes surrounded by fibrous tissue (F). Fig. 1f: higher magnification of fig. 1e showing regenerative hepatocytes (↑) surrounded by fibrous tissue (F) and inflammatory infiltrate (▲). Fig. 1g: group III showing central vein (CV), branched cords of liver cells with granular acidophilic cytoplasm and vesicular nuclei (↑) separated with congested hepatic blood sinusoids (▲). Fig. 1h: group III showing portal tract (PT) surrounded by mild inflammatory infiltrate (↑). Some hepatocytes showed small pyknotic nuclei (▲). (Fig.1a, 1b, 1c, 1d, 1f, 1g and 1h: x 400 – Fig. 1e: x 100)

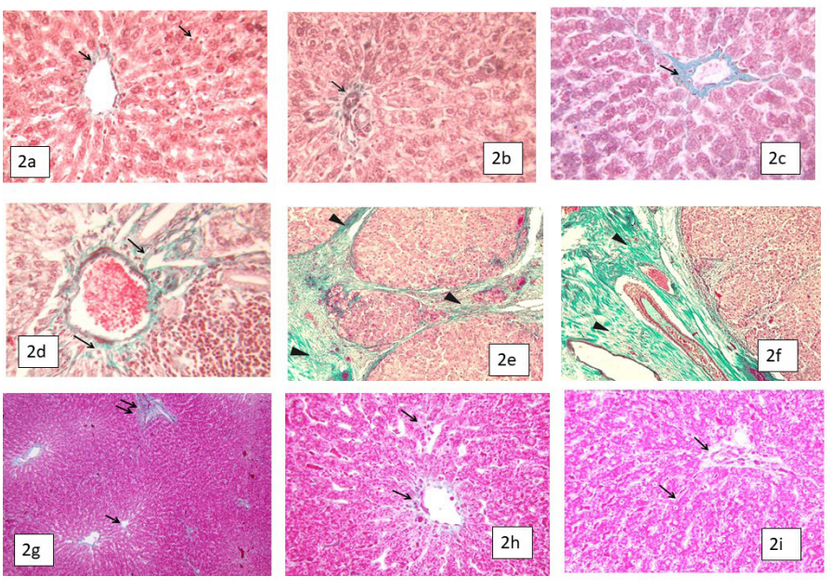


Fig. 2: Masson's trichrome-stained sections: Fig. 2a: control group revealed little amount of delicate collagen fibers (↑) encircling the central vein and in perisinusoidal space. Fig. 2b: control group showing few collagen fibers in the portal tract (↑). Fig. 2c and Fig. 2d subgroup IIA showing moderate increase in collagen fibers amount (↑) encircling the central vein and portal area (↑). Fig. 2e and Fig. 2f: subgroup IIB showing excessive deposition of collagen around nodules of regenerative hepatocytes (▲). Fig. 2g: group III showing few collagen aruond some central veins (↑) and some areas with increased collagen (↑↑). Fig. 2h and Fig. 2i: high power magnification of group III revealed little amount of collagen around the central vein, the portal tract and in perisinusoidal spaces. (Fig. 2a, 2b, 2c, 2d, 2f, 2h, 2i: × 400 - 2e, 2g: × 100)

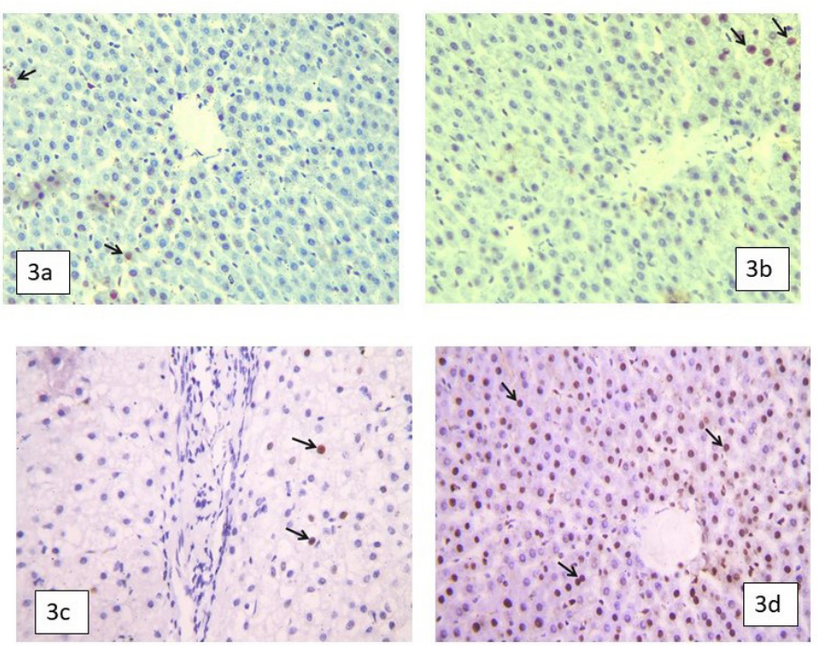


Fig. 3: PCNA stained sctions: 3a: Control group revealed few PCNA positive cells (↑). 3b: subgroup IIA revealed few PCNA postive cells (↑). 3c: subgroup IIB revealed few PCNA positive cells (↑). 3d: Group III revealed many PCNA positive cells (↑). (X400)

DISCUSSION

This study was designed to assess the effect of PRP on experimentally induced liver fibrosis in adult albino rat.

In the current study injection of CCL4 resulted in severe liver tissue injury including degeneration and necrosis of hepatocytes in the form of large vacuoles in the cytoplasm and small pyknotic nuclei. In addition to, congestion of blood vessels, dilatation of sinusoids, inflammatory infiltrate and finally fibrosis and loss of hepatic architecture. In agreement with these finding Hesami *et al*, 2016 demonstrated that CCL4 injection induced liver injury. Hepatocytes showed necrosis, vacuolations, inflammation and fibrosis.

They[13] explained that CCL4 is transformed to highly reactive trichloromethyle-free radical via cytochrome P-450, that affect the liver via lipid peroxidation. Then, cellular injury results when reactive oxygen species (ROS) rise in liver. These oxidants can damage cells by starting a chain of chemical reactions like lipid peroxidation or DNA oxidation that leads to damage of proteins and many components of the cells.

Masson's trichome stained sections revealed increased collagen fibers deposition in rats of both subgroup IIA and subgroup IIB. The results were proved by morphometric and statistical studies. Significant increase was found in the area percentage of collagen in both subgroups IIA & IIB as compared to control group (group I) and to PRP treated group (group III). Going with these finding[14,15,16] reported also increased collagen deposition in Masson's stained sections of livers of CCL4 treated rats.

The increase in collagen fibers was secondary to hepatic damage that activates HSCs which change to myofibroblasts that secrete collagen fibers. This is also explained by[17]. They mentioned that CCL4 causes oxidative stress that result in HSCs activation. Then, the activated HSCs become the main source of collagen and cytokine secretion. Recently, they mentioned that lipotoxic hepatocytes can stimulate Von-kupffer cells (KCs) that secrete transforming growth factor (TGF)-β1. Then, TGF-β1 stimulates HSCs which increase secretion and deposition of (hepatic collagen-α1) leading eventually to fibrosis. They added that collagen-α1 messenger RNA was markedly elevated in CCL4 injected mice while this elevation was prevented in TGF-β1-knockout mice. Moreover, interlukin-1β(IL-1β) and tumor necrosis factor-α (TNF-α) secretion by activated KCs was essential to keep HSCs existence via the nuclear factor-kappa (NF-kB) pathway[18].

In the present study, administration of PRP to CCL4-induced hepatic fibrosis model resulted into marked improvement in liver structure. This improvement appeared as significant decline in area percentage of collagen in comparison with both subgroups IIA and IIB. Also, significant increase in mean number of PCNA positive cells was reported. Some previous studies reported the same results[7,13,19].

The researchers[20] informed that PRP can result in liver regeneration through three mechanisms. The first one is direct effect of platelets; it happens when platelets is translocated to the perisinusoidal space. Then, they become in direct contact with hepatocytes and this triggers production of HGF, IGF-1 and VEGF. These growth factors (GFs) stimulate mitotic division in hepatocytes and stimulate liver proliferation. The second mechanism is involving liver sinusoidal endothelial cells; the direct contact between platelets and hepatic sinusoidal endothelial cells induces release of sphingosine-1-phosphate (SIP) from platelets, which stimulates production of interlukin-6 (IL-6) from the endothelial cells. Subsequently, IL-6 speeds up liver cells mitotic activity via IL6/STAT3 pathway. The third mechanism is including platelets and hepatic macrophage. Platelets accumulate and locally become activated in the liver by binding the surface of hepatic macrophage. This binding was confirmed by electron microscopy. Liver regeneration is stimulated by release of GFs from accumulated platelets and augmented via secretion of TNF-α and IL-6 from hepatic macrophage[20].

Other reseachers[19] demonstrated that PRP resulted in remarkable hepatic regeneration in an experimental model of fibrosis. Their results were proven by significant decrease in hepatic enzymes. Moreover, they reported that PRP decreased the expression of mRNA of fibrosis associated genes including TGF-β. In addition to, inflammatory related gene NF-kβ and liver IL-8. Moreover, they reported a significant increase in the anti-apoptotic marker Bcl-2 after PRP treatment, confirming PRP role in liver regeneration[19].

This study[21] stated that PRP has many growth factors with variable effects on hepatic fibrosis development. For example, platelet derived growth factor which has a profibrotic effect through activation of HSCs, whereas ATP, ILG-1 and HGF have antifibrotic effect through HSCs suppression. So, it is hard to understand and clarify the outcome of PRP administration[21].

Moreover, others[22] informed that PRP treatment increases the level of cAMP that has antifibrotic effect. This antifibrotic effect is mediated through Epac (Exchange proteins activated by cAMP). These Epac proteins adjust many cellular reactions through their capability to induce substitution of GTP for GDP on different G-proteins. Finally, cAMP reduces proliferation of fibroblasts, inhibits ECM protein synthesis by it and stimulates fibroblast apoptosis as well. They added that PRP has anti-inflammatory role which is facilitated via its impact on macrophage inflammatory proteins (MIP-1α) level in hepatic diseases. Increased HGF from PRP suppresses the phosphorylation and translocation of nuclear factor kappa B (NF-κB-p65) subunit to the nucleus resulting in suppression of pro-inflammatory genes expression. Consequently, PRP inhibits pro-inflammatory chemokines and the matrix metalloproteinase (MMPs) secretion[23].

CONCLUSION AND RECOMMENDATION

From the present study results, it is concluded that CCL4-induced liver fibrosis is not improved even after withdrawal of the causative drug. PRP injection improved the structure of liver after CCL4- induced fibrosis. Platelet therapy could offer a new approach for developing a new adjuvant therapy for treatment of liver diseases. More studies and clinical trials are recommended to determine the full potential and therapeutic effects of PRP in liver fibrosis and in other variety of indications.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Murata S, Maruyama T, Nowatari T, Taahashi K and Ohkohchi N. Signal transduction of platelet induced liver regeneration and decrease of liver fibrosis. Int. J. Mol. Sci. 2014; 15: 5412-5425.
2. El-Baz FK, Salama A, Salama RA. Therapeutic Effect of Dunaliella salina Microalgae on Thioacetamide-(TAA-) Induced Hepatic Liver Fibrosis in Rats: Role of TGF-β and MMP9. BioMed Research International. 2019 Sep 24: 1-9.
3. Poordad F. Review article: Thrombocytopenia in chronic liver disease. Aliment. Pharmacol. Ther. 2007; 26: S5-S11.
4. Porrett, P.M., Hsu, J. and Shaked, A. Late surgical complications following liver transplantation. Liver Transplant. 2009, 15, S12–S18.
5. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien PA. Platelet-derived serotonin mediates liver regeneration. Science. 2006 Apr 7;312(5770):104-7. doi: 10.1126/science.1123842. PMID: 16601191.
6. Murata S, Ohkohchi N, Matsu R, Ikeda O, Myronovych A and Hoshi R. Platelets promote liver regeneration in early period after hepatectomy in mice. World Journal of Surgery. 2007; 31(4): 808-816.
7. Hesami Z, Jamshidzadeh A, Ayatollahi M, Geramizadeh B, Farshad O and Vahdati A. Effect of Platelet-Rich Plasma on CCl4-Induced Chronic Liver Injury in Male Rats. International Journal of Hepatology 2014:1:1-7.
8. Everts P, Onishi K, Jayaram P, Lana JF, Mautner K. Platelet-Rich Plasma: New Performance Understandings and Therapeutic Considerations in 2020. International Journal of Molecular Sciences. 2020 Jan;21(20):7794.
9. Iredale J, Benyon R, Pickering J, McCullen M, Northrop M, Pawley S, Hovell C, Arthur MJ. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest. 1998: 102:538-54.
10. Kalbkhani M, Dehghani SN, Naji Haddadi S, Najafpour AR, Ghorbanzadeh N, Kalbkhani MH. Preventive role of platelet rich plasma in experimentally induced osteoarthritis in rabbits knee joint. Ash Ese Journal of medicine and medical researches. 2015;1(4):16-22.
11. Survarna SK, Layton C, Bancroft JD. bancroft's theory and practice of histological techniques, 7th edition, Churchill Livingstone, El Sevier 2013: 224, 408.
12. Sun H, Yu L, Wei H, Liu G. A novel antihepatitis drug, bicyclol, prevents liver carcinogenesis in diethylnitrosamine-initiated and phenobarbital-promoted mice tumor model. BioMed Research International. 2012 Jan 1;584728.
13. Hesami Z, Jamshidzadeh A, Ayatollahi M, Gramizadeh B, Vahdati A. The comparative effects of human mesenchymal stem cell and platelet extract on CCl4-Induced liver toxicity in rats. Platelets. 2016; 8: 1-9.
14. Liu F, Liu Z, Wu N, Cong X, Fei R, Chen H and Wei L. Transplanted Endothelial Progenitor Cells Ameliorate Carbon Tetrachloride–Induced Liver Cirrhosis in Rats. Liver Transplantation 2009: 15:1092-1100.
15. Hao T, Chen J, Zhi S, Zhang Q, Chen G, Yu F. Comparison of bone marrow vs. adipose tissue derived mesenchymal stem cells for attenuating liver fibrosis. Experimental and Therapeutic Medicine. 2017 Dec 1;14(6):5956-64.
16. De Luna-Saldivar M M, Marino-Martine IA, Franco-Molina MA, Rivera-Morales L G, Alarcón-Galvánd G, Cordero-Pérez P, Rojas-Martínez A, Rodríguez-Padilla C and Munoz-Espinosa L E. Advantages of adipose tissue stem cells over CD34+ mobilization to decrease hepatic fibrosis in Wistar rats. Annals of Hepatology. 2019: 18: 620–626.
17. Yang FR, Fang BW, Lou JS. Effects of Haobie Yangyin Ruanjian decoction on hepatic fibrosis induced by carbon tetrachloride in rats. World journal of gastroenterology: WJG. 2010 Mar 28;16(12):1458.-64.
18. Dou L, Shi X, He X, Gao Y. Macrophage phenotype and function in Liver disorder. Frontiers in Immunology. 2019; 10: 1-11.
19. Salem NA, Hamza A, Alnahdi H, Ayaz N. Biochemical and molecular mechanisms of platelet-rich plasma in ameliorating liver fibrosis induced by dimethylnitrosurea. Cellular Physiology and Biochemistry. 2018;47(6):2331-9.
20. Takahashi K, Murata S, Ohkohchi N. Platelet therapy: A novel strategy for liver regeneration, anti-fibrosis, and anti-apoptosis. World J Surg Proced. 2013 Nov 28;3(3):29-36.
21. SAMAR A, EL-ASWAD BE, MOHAMED AH, SHEREEN FM. Human Platelet Rich Plasma Alleviates Liver Fibrosis in Murine Schistosomiasis Mansoni. The Medical Journal of Cairo University. 2018 Dec 1;86(December):3807-23.

22. Shoeib HM, Keshk WA, Foda AM, Abo El Noeman SE. A study on the regenerative effect of platelet-rich plasma on experimentally induced hepatic damage in albino rats. Canadian journal of physiology and pharmacology. 2018;96(6):630-6.

23. Keshk WA, Zahran SM. Mechanistic role of cAMP and hepatocyte growth factor signaling in thioacetamide-induced nephrotoxicity: Unraveling the role of platelet rich plasma. Biomedicine & Pharmacotherapy. 2019 Jan 1; 109:1078-84.

الملخص العربى

التأثير النسيجي للبلازما الغنية بالصفائح الدموية على التليف الكبدى المستحدث برابع كلوريد الكربون فى الجرذ الأبيض البالغ

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المقدمة: تليف الكبد هو مشكلة صحية خطيرة تهدد الحياة في جميع أنحاء العالم. زرع الكبد هو العلاج الفعال الوحيد ، لسوء الحظ ، فهو محدود بسبب العديد من العقبات. لذلك ، هناك حاجة إلى علاجات بديلة لتليف الكبد. البلازما الغنية بالصفائح الدموية (PRP) هو اجراء قابل للتحدي لعلاج تليف الكبد.

الهدف من البحث: لتقييم تأثير البلازما الغنية بالصفائح الدموية على تليف الكبد المستحدث تجريبيًا في الجرذان البيضاء.

مواد وطرق البحث: تم استخدام أربعة وعشرين من الجرذان الإناث البالغات في هذه الدراسة. تم تقسيمها إلى ثلاث مجموعات: المجموعة الأولى: مجموعة ضابطة وشملت ٦ جرذان بالغة تلقت ٠,٥ مل من زيت الزيتون. المجموعة الثانية: تألفت من ١٢ جرذ. تم تقسيمها إلى المجموعة الفرعية الأولى : وشملت ٦ جرذان التي تلقت رابع كلوريد الكربون تم لمدة ستة أسابيع، وتم التضحيةبها بعد الحقن الأخيروذلك لاستحداث التليف، المجموعة الفرعيةالثانية: وشملت ٦ الفئران التي تركت للتعافي التلقائي لمدة أربعة أسابيع بعد الحقن الاخير برابع كلوريد الكربون . المجموعة الثالثة: تألّفت من ٦ جرذان كانت قد تم استحداث التليف بها ثم تم حقنها بالبلازما الغنية بالصفائح الدموية) ١مل/كجم) مرتين اسبوعيا لمدة أربعة أسابيع وبعدها تم التضحية بها. وقد تم تحضير عينات الكبد للصبغات النسيجية والهوستوكيميائية المناعية. كما تم عمل المقاييس الشكلية والتحليل الاحصائى .

النتائج: أدى حقن رابع كلوريد الكربون إلى إصابات متعددة في الكبد بما في ذلك الفجوات في السيتوبلازم والأنوية الصغيرة الداكنة. بالإضافة إلى ذلك ، أدى إلى تليف الكبد خاصة حول الوريد المركزي والمسالك الإلكترونية. العلاج بالبلازما الغنية بالصفائح الدموية أدى إلى تحسن واضح فى الأنسجة و في بنية الكبد ظهر ذلك في شكل السيتوبلازم الحمضي المحبب والأنوية النشطة في خلايا الكبد. وانخفاض كبير في تليف الكبد. لسوء الحظ، لم تتحسن مناطق قليلة في مجال الأنسجة الكبدية. مع زيادة كبيرة في المستضد الخلوى النووي المتكاثر.

الخلاصة: يمكن أن تكون البلازما الغنية بالصفائح الدموية علاجًا مساعدًا لتليف الكبد الذي يحتاج إلى مزيد من التحقيقات.