Effect of Curcumin on Collagen Deposition and Tissue Reorganization in Liver of Diabetic Rats

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Objective: *To localize and characterize type I and type IV collagens in recovery livers after curcumin supplementation in streptozotocin-induced diabetic rats.*
Material and Method: *Induced diabetic rats were performed by streptozotocin injection (60 mg/kg BW). Male rats were organized into three groups, control rat (C), diabetic rat (DM) and diabetic rat supplemented with curcumin (DMC) (200 mg/kg BW). At 8 weeks, animals were sacrificed. The localization and characterization of type I and type IV collagens in liver's cell and tissues were compared among C, DM and DMC groups by Sirius red and Immunohistochemical techniques, respectively.*
Results: *Type I and type IV collagens might be the key mediators of liver tissue healing associated with various disorders, especially with inflammation and reorganization processes. Concerning diabetic experiments, increased type I collagen was intensively recognized at subendothelial area of central veins whereas weakly demonstrated at periportal triad and perisinusoidal areas. Conversely, the high intensity of distribution of type IV collagen was strongly revealed at periportal triad and perisinusoidal areas while the intensity was faintly presented at central veins. In addition, accumulation of type IV collagen also revealed perisinusoidal basement membrane which was characteristic of capillarization of sinusoids. However, the localization of type I and type IV collagens was reduced after curcumin supplement in DMC rats compared with DM rats, implying that the liver tissue reorganization has been developed forwards to normal morphology. Moreover, type I and type IV collagen might distinctively accomplish the liver tissue reorganization by different means of area-based characterization.*
Conclusion: *The potential beneficial effect of curcumin has been exhibited the tissue reorganization of diabetic liver tissues. The efficiency and achievement of curcumin might be applied to be an alternative therapeutic agent in diabetic hepatic pathology.*

Keywords: *Curcumin, Diabetes, Liver injury, Type I & IV collagens, Tissue reorganization*

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Diabetes mellitus is a metabolic syndrome, which is one of the major worldwide health problems. According to diabetes, glucose metabolism is imbalance and lost homeostasis, leading to hepatic injury. Diabetes can cause long-term damages, leading to diabetic complications such as cardiovascular disease, nephropathy, retinopathy, neuropathy, fatty liver, steatohepatitis, and non-alcoholic fatty liver disease (NAFLD). Therefore, diabetes can seriously cause abnormalities of liver function, and finally liver failure[1,2].

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Hyperglycemic condition disrupts many cellular metabolisms, including carbohydrate, lipid and protein metabolisms and usually associates with oxidative environments of the cells and tissues. Moreover, fat accumulation and oxidative stress can influence diabetic pathology and complication, including diabetic liver damage. Reactive oxygen species generally damage cellular structures, such as cell membrane, proteins, and other intracellular molecules[3,4]. Enhancement of oxidative damage is related with many cellular environments, including cellular inflammation, extreme hyperglycemia, lipid peroxidation, and endoplasmic reticulum stress[5]. However, the cellular defense is usually activated to prevent the tissue damage.

Regarding hepatic injury, hepatic stellate cells, which located in subendothelial areas of central veins

and perisinusoidal space, are activated and behave as fibroblasts in order to synthesize collagen fibers in wound healing and repairing process. In general, collagens are associated and play roles in this process. However, deposition and localization of type I and type IV collagens are interested to be hallmarks during the healing process of diabetic liver tissue. Therefore, the appearance and detection of both types of collagen deposition in liver tissues might be the key markers for diagnosis[6,7]. Moreover, histological characterization and localization of type I and type IV collagens in diabetic liver tissue will reflect the patterns of area-based liver tissue reorganizations.

In general, hepatic sinusoids are lined and surrounded by fenestrated endothelium, without underlying continuous basement membrane. According to fibrin genesis, the construction of perisinusoidal basement membrane underneath this endothelium is formed and presented, termed as capillarization of hepatic sinusoids. Evidently, type IV collagen is one of the major components of hepatic perisinusoidal basement membrane[6,7].

Fascinatingly, curcumin or diferuloylmethane, a natural product extracted from rhizome of turmeric, has extensively studied in the effect of on the reduction of blood glucose levels[8]. Medical therapeutics of curcumin has been proposed for several biological activities, including anti-inflammation, antioxidant, and antifungal potential. Curcumin could attenuate the severity of hyperlipidemia and hyperglycemia conditions, hepatic disorder, nephropathy, pancreatitis, and also present hepatoprotective activity[9-11]. Moreover, its potential effect on wound healing and tissue repair has been progressively investigated. In curcumin-treated wounds, much more deposition of collagen was presented. Furthermore, cellular proliferation and synthesis of collagens in wound treatment by curcumin were healed and more rapidly improved as directed by enhanced rate of epitheliasation[12]. Moreover, it also has ability to eliminate oxygen-derived free radicals from the peroxidation of cellular lipids[12,13]. Therefore, spectrums of studies are suggested to study the efficiency of curcumin as a therapeutic agent for anti-diabetic actions.

Furthermore, Khimmaktong W et al[14] has proposed the formation of new blood vessels from preexisting vessels (angiogenesis), in diabetic liver tissue during pathophysiological conditions. The blood vessels of diabetic liver tissue treated with curcumin became regeneration and repair, directing to healthy and normal characteristics. Furthermore, the neovascularization of new nourishing blood vessels has considerably developed. Fundamentally, collagen fibers are one of the major portions of blood vessel's walls.

The purpose of this work is to evaluate the efficiency of curcumin on recovery of diabetic liver tissue. It is hypothesized that the curcumin's property would affect type I and type IV collagens production, accumulation and reformation. Therefore, curcumin may have potential to improve and repair damaged diabetic liver. Understanding the localization and characterization of each type of collagen will help to explain liver tissue reorganization in diabetic liver.

Material and Method

Preparation of diabetes animals

The study was carried on male Wistar rats (200-250 g), supplied by National Laboratory Animal Center of Mahidol University. To construct the model of insulin-dependent experimental model, streptozotocin (STZ) was used as a diabetogenic agent owing to its ability to destroy pancreatic beta cells[10,14]. Induced diabetic rats were performed by intraperitoneal injection of STZ (Sigma, St. Louis, MO, USA) (60 mg/kg BW) dissolved in citrate buffer while control group was injected with citrate buffer alone. Rats with blood sugar level >250 mg/dl were defined as diabetic animals. The animals were divided into three groups, control rats (C), diabetic rats (DM) and diabetic rats supplemented with curcumin (DMC) in corn oil daily (200 mg/kg BW, Sigma, St. Louis, MO, USA).

All animals were kept and maintained in controlled animal laboratory environment on alternative 12-hour light/dark periods (25±2°C), fed with standard rat chow and accessed ad libitum to water. The animal procedure has been approved by the Animal Care Committee, Faculty of Medicine, Srinakharinwirot University. At the end of 8-week-experimental design, the animals were sacrificed, then livers were collected and processed for paraffin blocks. Liver tissue sections were quickly excised and processed by Sirius red staining and immunohistochemistry techniques.

Sirius red staining

To investigate collagen accumulation, tissue samples were deparaffinized and rehydrated with xylene and series of ethanol, and then deionized with water, respectively. Liver tissues were stained with adequate Picro-Sirius Red stain kit (ab150681, Abcam, England) for 60 minutes. Then, tissues were rinsed in

two changes of acetic acid solution, followed by absolute alcohol. Liver tissues were counterstained with hematoxylin, and followed by tap water running. Finally, the sections were rehydrated in a series of ethanol, then cleaned with xylene and mounted by mounting medium, respectively.

Immunohistochemistry for type I and type IV collagen

The liver tissue sections on positive charged coated slide: control rats (C), diabetic rats (DM) and diabetic rats supplemented with curcumin (DMC), were cleared in series of xylene, followed by rehydration in graded series of ethanol. Concerning antigen retrieval, the tissue sections were immersed in ethylenediaminetetraacetic acid (EDTA) at 70°C for 10 minutes and then washed in distilled water 2 times.

Next, the liver tissue sections were blocked for endogenous peroxidase by 3% hydrogen peroxide (H_2O_2) in absolute methanol for 50 minutes, washed in distilled water and dipped into phosphate buffer saline plus tween-20 (PBS-T). The sections were then incubated with 0.1% glycine in phosphate buffer saline (PBS) in moist chamber. To accomplish non-specific binding protein suppression, the sections were subsequently incubated with 1.5% blocking serum in PBS for 60 minutes (reagent: Santa Cruz, CA, USA).

Both primary antibodies: anti-collagen type I (SC-25974) and anti-collagen type IV (SC-18178) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). The first primary antibodies was goat polyclonal antibody raised against type I collagen protein and the other one is a goat polyclonal antibody raised against type IV collagen protein. The process was performed at 1: 50 dilution concentration of primary antibodies in 1.5% blocking serum at 4°C for overnight, and then washed in PBS-T.

The sections were further incubated with biotinylated secondary antibody (SC-2023) at dilution 1: 500 at room temperature for 60 minutes and washed in PBS-T. Then, the sections were incubated with Avidin-biotin complex for 30 minutes and washed in PBS-T. Peroxidase conjugate were subsequently localized by 3, 3'diaminobenzidinetetrahydrochloride (DAB) (reagent: Santa Cruz, CA, USA) as a chromogen. Then, the sections were cleared in distilled water and counterstained with Mayer's hematoxylin. Finally, dehydration of tissue sections was performed by continuously dipping into a series of alcohol. Sections were further cleansed by dipping into series of xylene and protected by mounting medium together with cover slips. The investigation of immunohistochemistry results were examined and photographed by light microscope (BX-50, Olympus, Japan). Negative control was performed in parallel with experimental procedure by replacing the primary antibody with phosphate buffer saline.

Results

Collagen accumulation in liver parenchyma

In order to detect the density and localization of collagen accumulation, histological visualization of collagen was performed by Sirius red staining kit, which specifically distinguished collagen in red color.

Characterization and localization of collagen were demonstrated and compared among C, DM, and DMC groups (Fig. 1-3). In control group, collagen was weakly recognized in liver parenchyma (Fig. 1A, 1D, 2A, 2D, 3A, 3D). According to DM group, the large amount of collagen accumulation and deposition were spectacularly revealed and distributed as strong as red color intensity all over the liver parenchyma. Regarding collagen localization, collagens were surrounded at central veins, especially at subendothelial areas (Fig. 2B, 2E), at periportal triad areas (Fig, 1B, 1E), and also presented along the hepatic sinusoids (perisinusoidal space) (Fig. 3B, 3E). As noted, the presence of collagen and capillarization of sinusoids are ones of the markers for liver tissue reorganization.

After curcumin supplementation in DMC group, the collagen localization turned out to very weak intensity of red color (Fig. 1C, 1F, 2C, 2F, 3C, 3F). Identification of collagens was still demonstrated at central veins and some areas of perisinusoid. The intensity and pattern of collagen distribution were recovered and appeared similarly to the control group.

Effect of curcumin on type I collagen immuno-reactivity in liver tissues among C, DM, and DMC groups

In control groups, the presentation of type I collagen was infrequently observed. Only some spots of brownish color were scattered in liver parenchyma, at central vein and perisinusoidal space (Fig. 4), respectively.

Regarding DM groups, immunohistochemical marker demonstrated that type I collagen was continuously recognized as very strong intensity around the central veins, especially at subendothelial areas (Fig. 5A, 5D). Moreover, hepatocyte surrounding the central veins, which might be activated hepatic stellate cell (HSC), also revealed collagen accumulation in cytoplasm which was distinguished prominently from

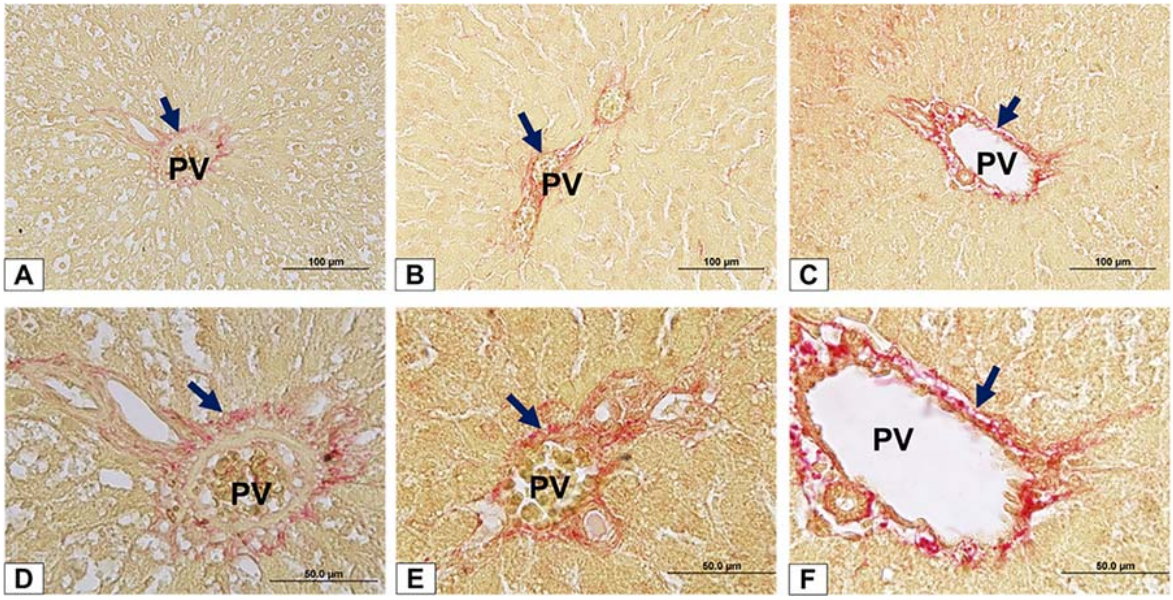

Fig. 1 Micrographs of collagen accumulation in periportal triad of liver tissue at 8 weeks, by Sirius red staining. Control group (A* and D**) showed very low intensity of collagen (arrow). Hepatocytes arranged as normal regular hepatic cord. DM group (B* and E**) exhibited intensity of collagen distributed around periportal triad (arrow). DMC group (C* and F**) presented the decreasing of accumulation of collagen (arrow).
*40X magnification; **100X magnification; PV = portal vein

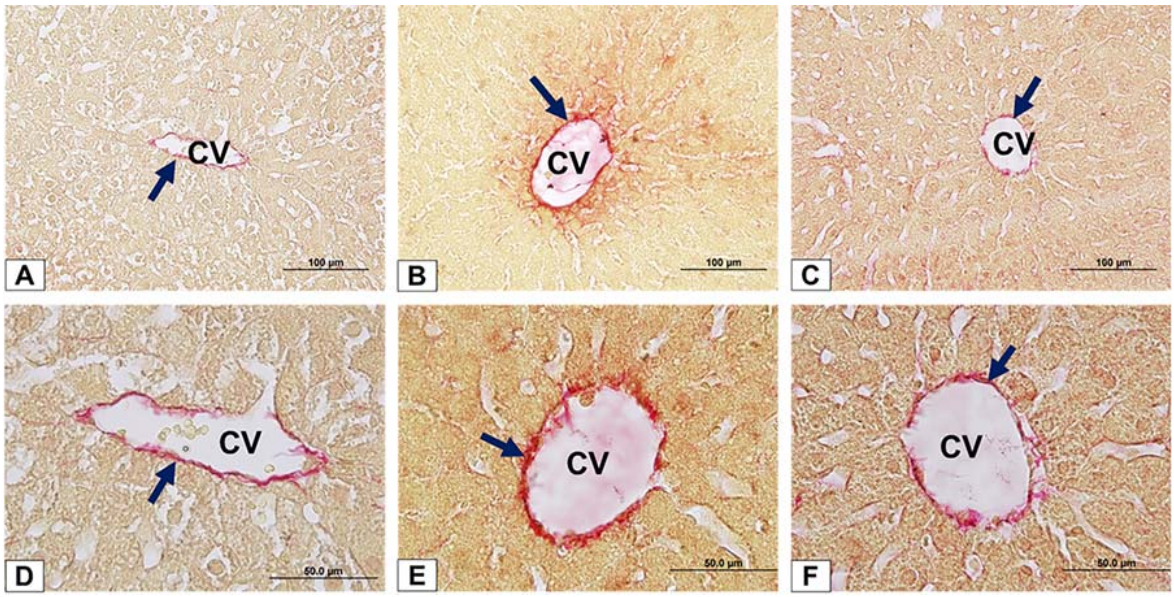

Fig. 2 Micrographs of collagen accumulation in central vein of liver tissue at 8 weeks, by Sirius red staining. Control group (A* and D**) showed very thin intensity of collagen (arrow). DM group (B* and E**) exhibited strong intensity of collagen distributed around central vein (arrow). DMC group (C* and F**) presented the reduction of collagen accumulation (arrow).
*40X magnification; **100X magnification; CV = central vein

the control. However, increased accumulation of type I collagen was additionally demonstrated medium intensity at periportal triads and low intensity at perisinusoidal spaces (Fig. 5B-C, 5E-F). Therefore, the

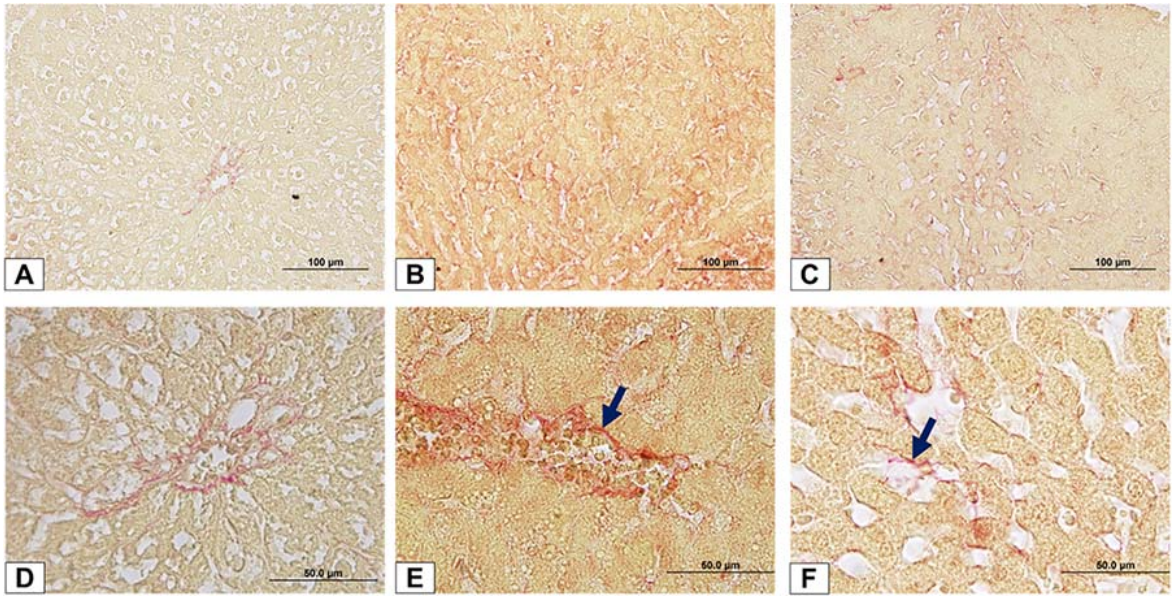

Fig. 3 Micrographs of collagen accumulation in perisinusoid of liver tissue at 8 weeks, by Sirius red staining. Control group (A* and D**) revealed intensity of collagen that was very less or not found red color. DM group (B* and E**) exhibited dense intensity of collagen distributed densely along with perisinusoid (arrow). DMC group (C* and F**) existed the lessening of red color of collagen deposition (arrow).
*40X magnification; **100X magnification

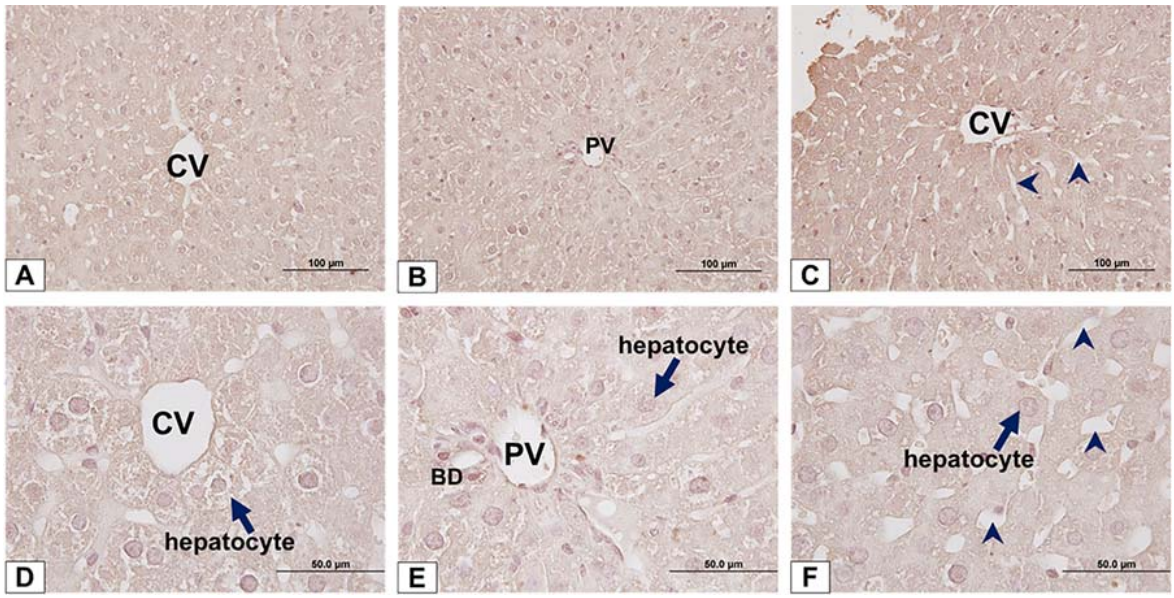

Fig. 4 Micrographs of type I collagen accumulation in control group of liver tissue at 8 weeks, by immunohistochemical staining. Some spots of brownish color were spread in liver parenchyma at central vein (A* and D**), portal triad (B* and E**) and perisinusoidal space (C* and F**).
*40X magnification; **100X magnification; Arrow head = Sinusoidal space; CV = Central vein; PV = Portal vein; BD = Bile duct

greater to lower levels of type I collagen intensity was exhibited at HSC surrounding the central veins, especially at subendothelial areas, periportal triads and perisinusoidal spaces, respectively. It might be

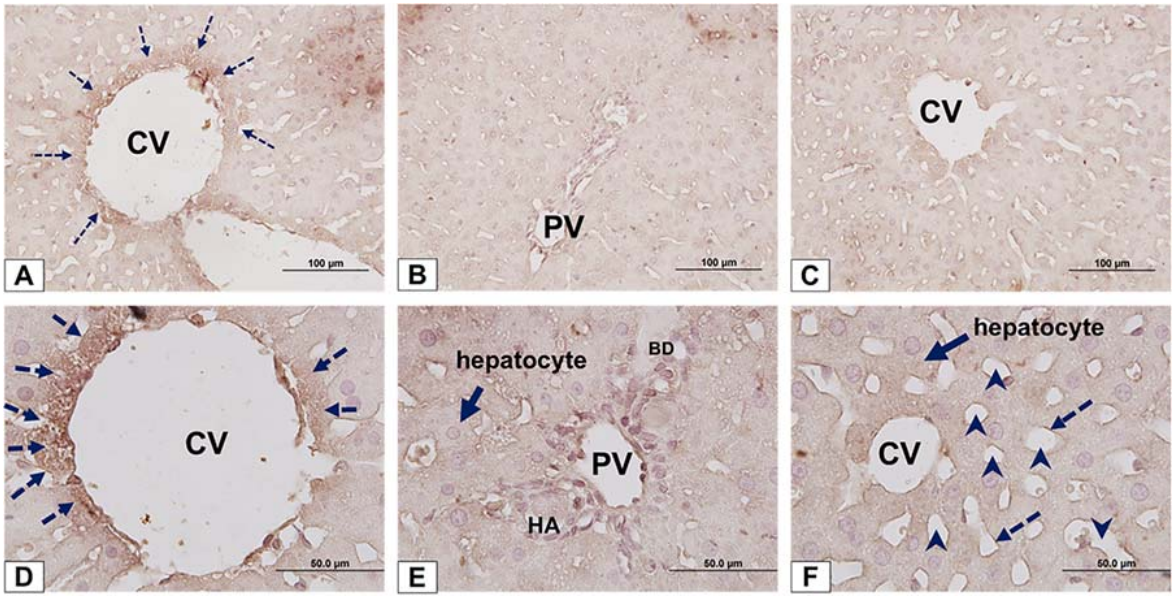

Fig. 5 Micrographs of type I collagen accumulation in DM group of liver tissue at 8 weeks, by immunohistochemical staining. The intensity of brownish color were spread strongly around the central vein and hepatocyte underneath the central vein (A* and D**). Type I collagen were accumulated in moderate intensity at portal triad (B* and E**) and low intensity at perisinusoidal space (C* and F**).
*40X magnification; **100X magnification; Dashed arrow = Type I collagen; Arrow head = Sinusoidal space; CV = Central vein; PV = Portal vein; HA = Hepatic artery; BD = Bile duct

suggested that identification and accumulation of type I collagen were principally developed surrounding the central veins, especially at subendothelial areas.

In contrast, after curcumin supplementation in DMC group, the accumulation and deposition of type I collagen were obviously reduced and fade out. The restoration and recovery of hepatic parenchyma were reflected closely similar to the control group as shown in Fig. 6. It might be suggested that liver tissue reorganization was performed effectively by curcumin. Type I collagen should reform themselves according to the process of tissue reorganization and healing process.

Effect of curcumin on type IV collagen immunoreactivity in liver tissues among C, DM, and DMC groups

Approaching to control group, localized type IV collagen was hardly recognized along the hepatic parenchyma (Fig. 7). In contrast, type IV collagen in DM group was intensively evidenced by distributing brownish color all over the liver tissue (Fig. 8). Specifically, the perisinusoidal area revealed very strong brownish intensity of type IV collagen accumulation (Fig. 8C, 8F). It might be suggested that perivascular areas of sinusoid accumulation of type IV collagen along the hepatic plate might reflected the stages of collagen performance and capillarization. It might be proposed that type IV collagen played an important role in liver tissue reorganization, specifically at the perisinusoidal areas along the hepatic plates. In addition, type IV collagen is the major component of basement membrane of many structures. Therefore, liver tissue reorganization at sinusoid areas might require the activity and responsibility of type IV collagen.

Comparing to type I collagen intensity in DM group, the medium intensity level of identification and accumulation of type IV collagen was observed around the periportal triads whereas the low level intensity of type IV collagen was demonstrated as punctate spots in the subendothelial areas of central veins (Fig. 8A, 8D). It might be suggested that type IV collagen may not play important roles for tissue reorganization at the areas of periportal triads and subendothelial areas of central veins.

In supplementation of curcumin in DMC group, the intensity of brownish color presented type IV collagen was generally decreased in hepatic parenchyma area (Fig. 9A, 9D). Only weak immunoreactivity was dispersed in some areas of central

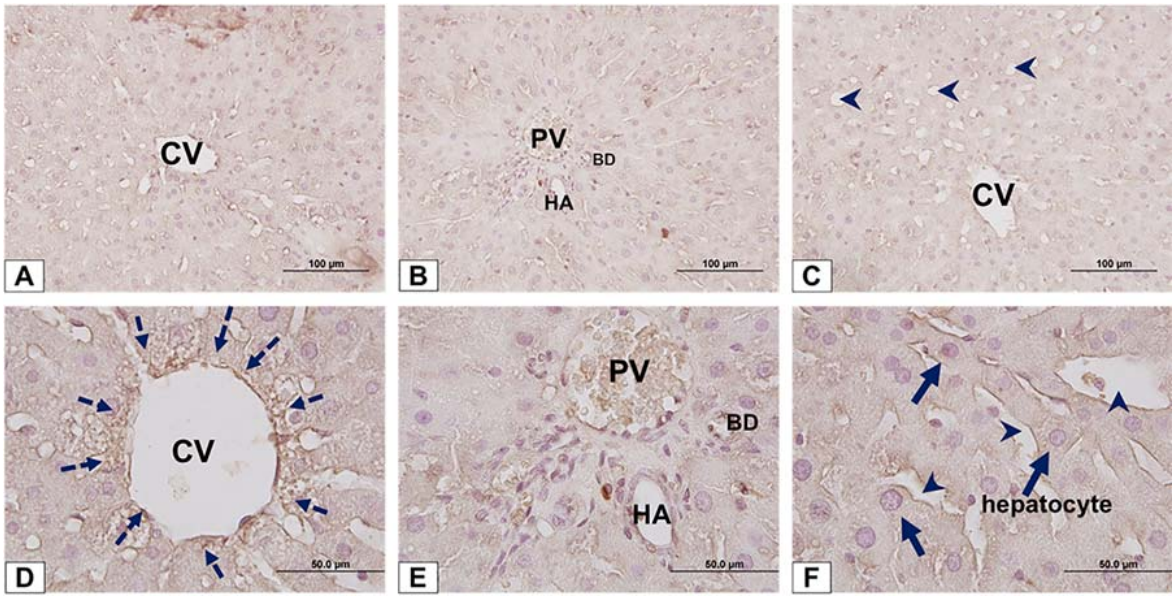

Fig. 6 Micrographs of type I collagen accumulation in DMC group of liver tissue at 8 weeks, by immunohistochemical staining. The intensity of type I collagen were reduced both central vein (A* and D**), periportal triad (B* and E**), and sinusoid (C* and F**) when compared with type I collagen in DM group.
*40X magnification; **100X magnification; Dashed arrow = Type I collagen; Arrow head = sinusoidal space; CV = Central vein; PV = Portal vein; HA = Hepatic artery; BD = Bile duct

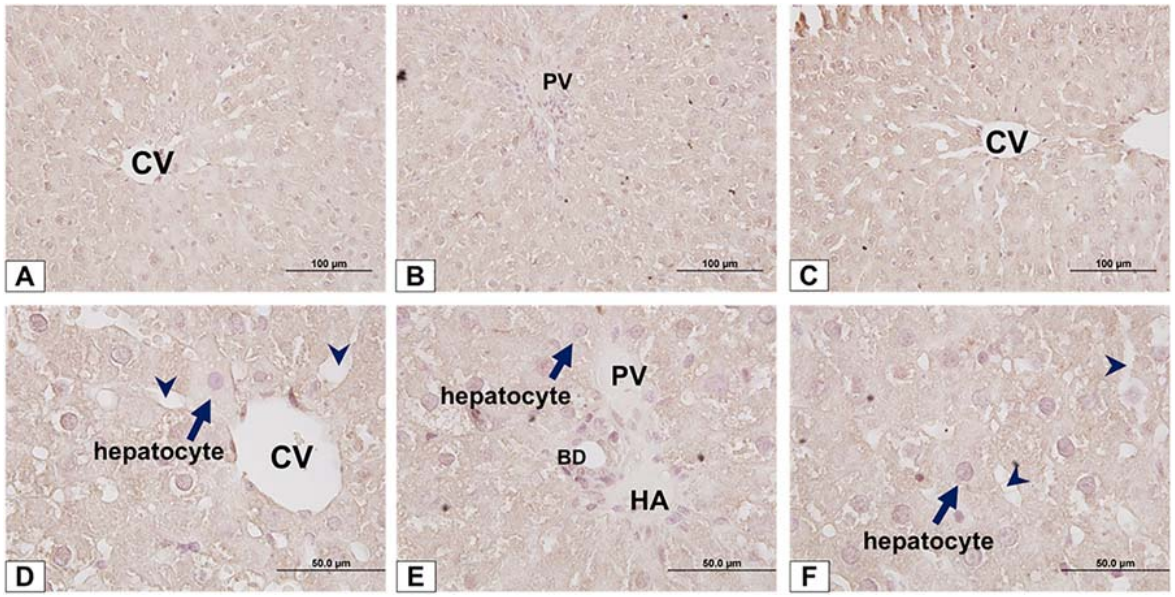

Fig. 7 Micrographs of type IV collagen accumulation in control group of liver tissue at 8 weeks, by immunohistochemical staining. Brownish color of type IV collagen was barely recognized all of central vein (A* and D**), periportal triad (B* and E**), and perisinusoid (C* and F**).
*40X magnification; **100X magnification; Arrow head = Sinusoidal space; CV = Central vein; PV = Portal vein; HA = Hepatic artery; BD = Bile duct

veins, periportal triads, and perisinusoidal areas. In addition, the recovery and reform of liver tissues were evidently decreased accumulation of type IV collagen, demonstrating by very low intensity of immunostaining

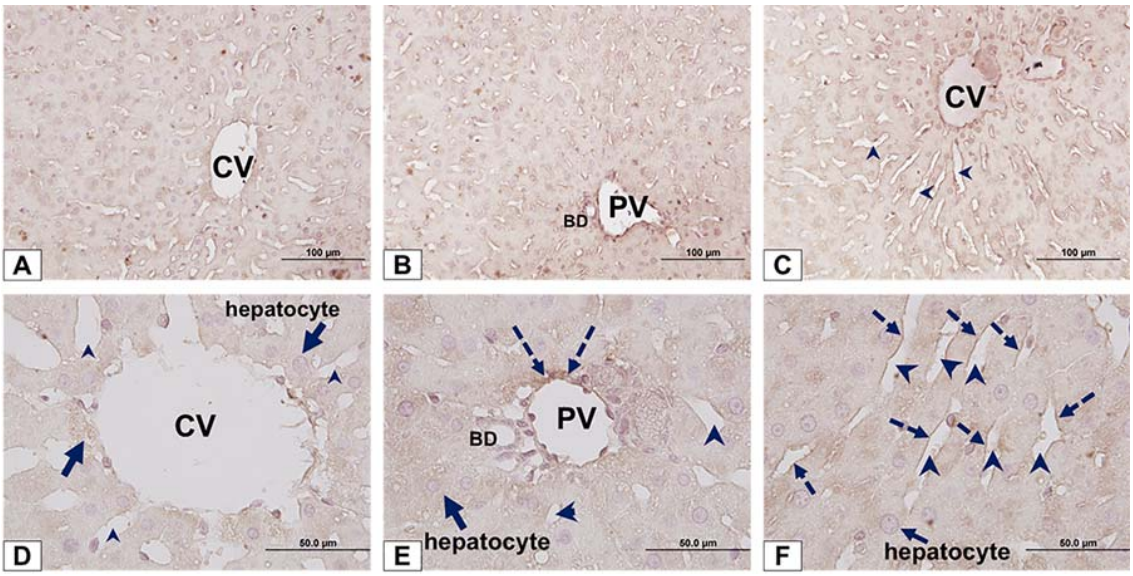

Fig. 8 Micrographs of type IV collagen accumulation in DM group of liver tissue at 8 weeks, by immunohistochemical staining. The accumulation of type IV collagen at central vein (A* and D**) was less intensity. In contrast, type IV collagen deposition was observed in the perisinusoid (C* and F**) and moderate intensity was found at periportal triad (B* and E**).
*40X magnification; **100X magnification; Arrow dashed = Type IV collagen; Arrow head = Sinusoidal space; CV = Central vein; PV = Portal vein; BD = Bile duct

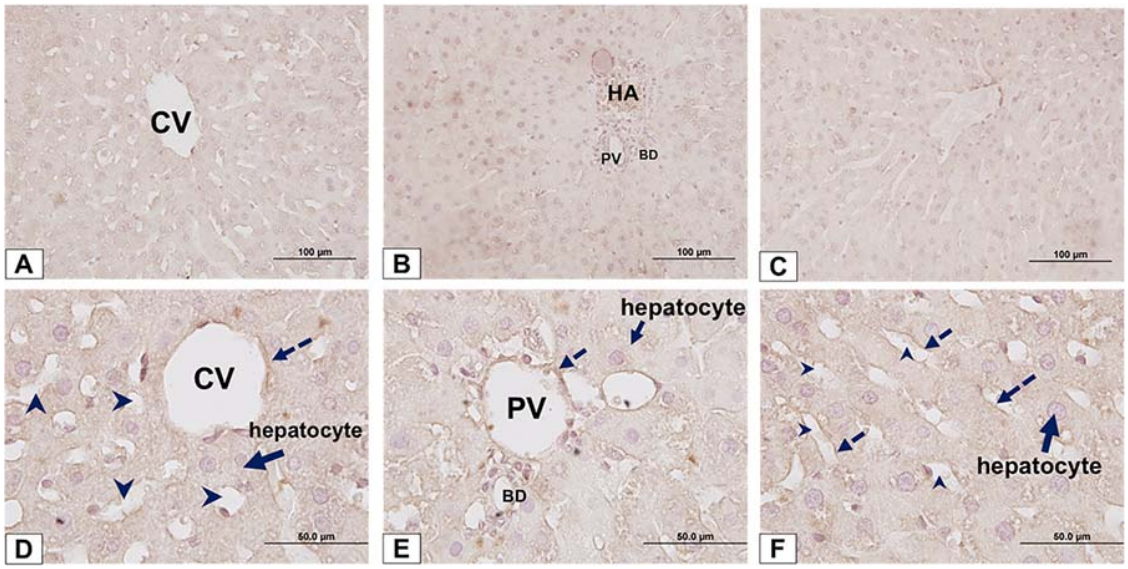

Fig. 9 Micrographs of type IV collagen accumulation in DMC group of liver tissue at 8 weeks, by immunohistochemical staining. The intensity of type IV collagen was decreased in central vein (A* and D**), periportal triad (B* and E**), and sinusoid (C* and F**) when compared with type IV collagen in DM group (Fig. 8).
*40X magnification; **100X magnification; Dashed arrow = Type IV collagen; Arrow head = Sinusoidal space; CV = Central vein; PV = Portal vein; HA = Hepatic artery; BD = Bile duct

(Fig. 9). Therefore, liver tissue reorganization, especially at the perivascular areas of hepatic sinusoids should be predominantly repaired and performed by the activity of type IV collagen.

Analysis for type I and type IV collagen accumulation in the liver

The immunoreactivity of type I and type IV collagens in liver tissues of C, DM, and DMC groups were evaluated and scored as shown in Table 1.

Discussion

Concerning control group, collagen appearance was rarely seen as fundamental pattern in hepatic parenchyma. In contrast in diabetic condition, type I collagen accumulation and deposition were mostly recognized continuously surrounding central veins, especially at subendothelial areas whereas type IV collagen accumulation and distribution were mainly observed along perisinusoidal space of hepatic plates. Liver damage is initiated and stimulated by liver injury through various cellular events, including necrosis, apoptosis, inflammation, tissue remodeling and repair processes together with extracellular matrix modification[6,7,15]. Liver regeneration related with liver fibrinogenesis is interrelated to extracellular matrix modifications by accumulation and deposition of large numbers of collagen components in liver parenchyma. Proteins production and release during extracellular modification and remodeling are important as key markers to validate the stages of liver damage. Two types of collagens, type I collagen and type IV collagen, were focused in this work.

Pro-peptide and mature of type I collagen were proposed to associate with the feature of liver reorganization. Type I collagen presented predominant deposition in the extracellular matrix in wounded liver[7,]. However, type I collagen was also declared to be the major component of connective tissue and also associated with both mild liver diseases and moderate severe liver destructions[6,16]. In DM liver tissue, high levels of elevated type I collagen were principally observed, presenting accumulation and deposition as continuous layer surrounding central veins, especially at subendothelial areas. It might be suggested that activated hepatic stellate cells (HSCs) around central veins were triggered to change responsibility in order to promote the production of type I collagen. Then, the selected remodeling of connective tissue might associate to recover the stages of liver injury.

According to liver damage, some reports suggested that HSCs play pivotal roles in liver disease and pathogenesis. In pathological condition, injured hepatocyte and immune cells send signals to HSCs, then quiescent HSCs can be triggered to become activated HSCs, then they changed and transdifferentiated to be activated fibroblasts or myofibroblasts. At injured liver areas, the activated HSCs could produce extracellular matrix (ECM) elements composed of collagen, proteoglycan, and adhesive glycoproteins to protect the liver. Furthermore, activated HSCs also secrete growth factors and cytokines to stimulate the hepatocyte regeneration[17]. Moreover, HSCs are also stimulated by other cells, including hepatocyte, T-lymphocyte, and Kupffer cells. The systems of stimulation are groups of cytokine and inflammatory secretions such as transforming growth factor beta (TGF-beta), tumor necrosis factor alpha (TNF-alpha), insulin-like growth factor (IGF), interleukin-6 (IL-6), interferon gamma (INF-gamma), etc[6,7,18].

In previous reports, type IV collagen is the major component of basement membrane protein. Increased formation of continuous perisinusoidal basement membrane, which was constructed as feature of capillarization of sinusoids, indicated stages of liver reorganization[15]. In addition, type IV collagen is important as a recognized marker of basement membrane construction and capillarization of sinusoids. The pathology of liver might have some effects linked with the production and accumulation of type IV collagen,

Table 1. The scored intensity of type I and type IV collagens deposition among C, DM, and DMC groups

Collagen deposition areas	Type I collagen			Type IV collagen		
	C	DM	DMC	C	DM	DMC
Central vein	+	++++	++	+	+	+
Subendothelial area						
Periportal triad	+	++	+	+	+++	+
Perisinusoidal space	+	++	+	+	++++	++

- = No intensity; + = Very weak intensity; ++ = Weak intensity; +++= Moderate intensity; ++++ = High intensity

as proposed in some liver diseases and hepatocellular carcinoma[19].

In animal model of liver damage, increased pro-peptide of type IV collagen was detected in the bile duct ligation and carbon tetrachloride induction[16]. In addition, Fallatah[6] has proposed that type IV collagen is the important surrogate maker of liver fibrogenesis. The increased level of type IV collagen is correlated significantly with the hepatic reorganization. Regarding DM condition, type IV collagen was distinctly localized in the perisinusoid of hepatic plate, along the periseptal parenchyma, demonstrated as punctate spots around central veins. This complication might accompany with the accumulation and deposition of type IV collagen, correlating with the feature of liver reorganization and recovery.

However, the low levels of both type I and type IV collagens localization and characterization in DMC group could be implied that curcumin could improve liver tissues destruction, demonstrating by tissue repair and remodeling. On the contrary, the increase in collagen level in the DM liver tissue suggested that hyperglycemia might stimulate inflammatory situation to hypoxia and increased expression of collagen for liver tissue remodeling.

About herbal remedy, curcumin has been shown to reduce blood glucose levels in diabetes. Curcumin, a polyphenol component from rhizome of turmeric, demonstrates potent biological activities, including antioxidant, anti-inflammation, anticancer, antifungal, and wound healing[12,13,20,21]. Additionally, the beneficial therapeutic effect have been reviewed for treatment of diabetes and diabetic complications, including nephropathy, neuropathy, vascular diseases, adipose tissue dysfunction, and liver disorders. In diabetes-associated disorder, curcumin plays protective roles against oxidative damage by scavenging of reactive oxygen species, modulating hepatic metabolism and antioxidant enzymes[12-14,20,22].

Generally, wound healing and tissue restoration are complicated and involved in many events, including inflammation, granulation, and tissue remodeling. Curcumin-treated wounds have demonstrated reepithelialization, increased migration of many cells such as macrophages, fibroblast, and myofibroblast, neovascularization, improved collagen synthesis and deposition. In curcumin-treated wound, transforming growth factor beta 1 (TGF-beta 1) and fibronectin were considerably increased. Actually, TGF-beta 1 plays important role to enhance wound healing. Therefore, curcumin has been proposed to improve wound healing by modulating TGF-beta 1[9,22]. Consequently, it might be implied that curcumin might act for potential treatment to modulate and regulate liver tissue remodeling and reorganization by means of liver tissue healing and repair.

Conclusion

It is possibly proposed that curcumin could improve the destruction of the liver tissues, by remodeling of type I and type IV collagens accumulation and deposition. Potential treatment with curcumin in diabetes has been demonstrated meaningfully about the therapeutic consequence in improvement and recovery of liver reorganization. The efficiency and achievement of curcumin might be applied to be an alternative novel therapeutic agent in diabetic hepatic pathology.

What is already known on this topic?

In diabetes mellitus, the liver tissue complications are related to inflammation and tissue destruction. Many pro-inflammatory cytokines and related cellular responses have been widely studied. Regarding histological study, one of the cellular and tissue responses is the formation of extracellular matrix, especially collagen. However, there are many types of collagen which also have different functions and accumulation among various tissues. It is suggested that collagen formation and deposition in diabetic tissues are one of the important factor to redevelop or reorganize injured tissues.

What this study adds?

This study has intended to demonstrate the beneficial efficacy of curcumin on liver complication in diabetic animal model. Regarding diabetes, liver tissue undergoes inflammation and further morphological destruction. Liver tissue supplemented with curcumin reveals recovery and reorganization by the effort of both collagen type I and type IV. However, localization and characterization between collagen type I and type IV are demonstrated at different areas of liver tissues. The potential effect of curcumin has demonstrated the liver tissue recovery and reorganization of diabetic liver tissues. The competence and accomplishment of curcumin might be interested to apply in alternative medicine to attenuate liver diabetic complications.

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Potential conflicts of interest

None.

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ผลของ curcumin ต่อการสะสมของเส้นใยคอลลาเจนและการฟื้นฟูของเนื้อเยื่อตับในหนูที่เป็นเบาหวาน

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วัตถุประสงค์: เพื่อศึกษาผลของสาร curcumin ที่มีต่อการสะสมของ collagen type I และ type IV และการฟื้นฟูของเนื้อเยื่อตับในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานโดยสาร streptozotocin (STZ)

วัสดุและวิธีการ: หนูถูกเหนี่ยวนำให้เป็นเบาหวานโดยสาร STZ (60 mg/kg BW) หนูเพศผู้ถูกแบ่งออกเป็น 3 กลุ่ม ได้แก่หนูกลุ่มควบคุม กลุ่มเบาหวาน และกลุ่มเบาหวานที่ได้รับสาร curcumin (200 mg/kg BW) เป็นระยะเวลา 8 สัปดาห์ ได้ศึกษาลักษณะโครงสร้างของเนื้อเยื่อตับ เน้นที่การสะสมของ collagen type I และ type IV และการฟื้นฟูของเนื้อเยื่อตับในหนูของหนูทั้ง 3 กลุ่มโดยวิธี immunohistocehemistry

ผลการศึกษา: Collagen type I และ type IV เป็นสารตัวกลางที่สำคัญในการซ่อมแซมฟื้นฟูเนื้อเยื่อตับที่มีการถูกทำลาย โดยมีความเกี่ยวข้องต่อเนื่องกับกระบวนการอักเสบและการปรับตัวเพื่อฟื้นฟูเนื้อเยื่อในตัวอย่างกลุ่มเบาหวานพบว่ามีการสะสมของของ collagen type I เป็นจำนวนมากที่บริเวณ subendothelial area of central vein และ peri-portal triad และมีเป็นจำนวนน้อยที่บริเวณ perisinusoidal area ส่วน collagen type IV พบว่ามีการสะสมเป็นจำนวนมากที่บริเวณ peri-portal triad และ perisinusoidal area และมีเป็นจำนวนน้อยที่บริเวณ central vein การสะสมและการเพิ่มปริมาณของ collagen type IV ที่บริเวณ perisinusoidal area น่าจะมีบทบาทไปถึงการสร้างและฟื้นฟู perisinusoidal basement membrane ซึ่งส่งผลต่อการซ่อมแซมและฟื้นฟู sinusoid ส่วนกลุ่มทดลองที่เป็นเบาหวานและได้รับสาร curcumin พบว่ามีการลดลงของการสะสมของ collagen type I และ type IV และมีสภาพเข้าสู่สภาวะปกติดังนั้น collagen type I และ type IV มีบทบาทต่อการซ่อมแซมและฟื้นฟูเนื้อเยื่อตับในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานโดยมีการปรากฏและสะสมในรูปแบบและบริเวณที่แตกต่างกัน

สรุป: สาร curcumin มีบทบาทในการฟื้นฟูและซ่อมแซมเนื้อเยื่อตับในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานโดยสามารถศึกษาการแสดงออกของ collagen type I และ type IV ร่วมกับพยาธิสภาพในเนื้อเยื่อตับดังนั้น curcumin จึงมีประสิทธิภาพและคุณค่าที่น่าจะนำไปประยุกต์ใช้เป็นสารในการรักษาป้องกันในการพัฒนาการรักษาพยาธิสภาพของตับในโรคเบาหวานต่อไป