ORIGINAL ARTICLE

Antifibrotic effect of heparin on liver fibrosis model in rats

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Abstract

AIM: To evaluate the effect of chronic thrombin inhibition by heparin on experimentally induced chronic liver injury (liver fibrosis) in rats.

METHODS: Chronic liver injury (liver fibrosis) was induced in Wistar rats by oral administration of carbon tetrachloride (CCl_4) for 7 wk, an animal model with persistent severe hepatic fibrosis. Intravenous administration of the thrombin antagonist (heparin) started 1 wk after the start of CCl_4 intoxication for 6 wk. After completion of treatment (7 wk), markers of hepatic dysfunction were measured and changes evaluated histopathologically.

RESULTS: Higher serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total, direct and indirect bilirubin levels, as well as lower fibrinogen levels, were found in CCl_4 intoxicated rats. Heparin, silymarin and combination of drug (heparin and silymarin) treatment for 6 wk prevented a rise in SGOT, SGPT, ALP, total, direct and indirect bilirubin levels and improved fibrinogen levels. Deterioration in hepatic function determined by the fibrosis area was retarded, as evident from hepatic histopathology. Total protein levels were not changed in all groups.

CONCLUSION: Heparin, a thrombin antagonist, preserved hepatic function and reduced severity of hepatic dysfunction/fibrogenesis. Combination of heparin and silymarin produced additional benefits on liver fibrosis.

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INTRODUCTION

Liver fibrosis is one of the main complications of chronic liver disease. It is insidious in nature as symptoms appear late. Liver fibrosis is characterized by an excessive deposition of extracellular matrix (ECM) components in the liver parenchyma. The treatment of liver fibrosis or cirrhosis is always a problem in clinical practice. To control and stop liver fibrosis towards cirrhosis is of utmost importance. Although liver fibrosis is known to be a serious chronic liver disease, an ideal agent to be used in this disease is still not available and there has been a constant urge for the discovery of newer drugs.

During the past decade, it was understood that activation of the coagulation cascade classically proceeds and eventually leads to cirrhosis. If not detected and treated in time, there is imminent danger of permanent liver failure. Synthesis of ECM components is performed by fibrogenic cells that derive from the activation of precursors, such as hepatic stellate cells and fibroblasts. During the past decade, a lot of attention has been given to the

stimuli responsible for fibrogenic cell activation in the liver[1].

The role of the coagulation cascade has been suggested by various observations but has not been formally proved. Activation of the coagulation cascade classically proceeds through the activation of thrombin. Thrombin is responsible for the conversion of fibrinogen to fibrin. Fibrin accumulates in the liver during acute and chronic experimental liver injury[2]. A similar result has been observed in models of pulmonary fibrosis[3]. Clot formation by itself leads to flow disturbance and local hypoxia which is a cofactor for fibrosis[4]. Thrombin signals *via* specific cellular receptors to regulate functions associated with tissue remodeling[5]. Thrombin receptor protease activated receptors (PAR)-1 is up regulated in human liver during acute and chronic injury[6]. Thrombin stimulates the proliferation of rat and human hepatic stellate cells.

Recently, thrombin inhibitors have attracted attention as a new class of drugs for the treatment of liver fibrosis. Direct inhibition of thrombin with a synthetic antagonist decreased lung collagen accumulation in experimental pulmonary fibrosis[7]. There are no clear data for liver fibrosis. Moreover, heparin could inhibit the growth of the Ito cells effect *in vitro*, which suggested that heparin does display some thrombin inhibitory activity[8]. A role for thrombin in liver fibrosis has been reported[9]. Hence, chronic thrombin inhibition decreases the rate of thrombin deposition and thus reduces liver injury. There are few reports suggesting beneficial effects of thrombin antagonism in liver fibrosis[10].

However, no attempt has been made to come to a logical conclusion about the relative beneficial effects of such drugs in liver fibrosis. Furthermore, heparin has been reported to inactivate the coagulation system and prevent acute liver injury from a hepatotoxic dose of lipopolysaccharide[10]. Similarly, heparin has been effective in cholestatic liver injury in rats[11]. Moreover, the effects of heparin on liver fibrosis in patients with chronic hepatitis B has been reported[12].

In the light of the above and looking at the beneficial properties of heparin in liver disorders, the present study was performed to investigate the therapeutic potential of heparin by studying its effect in an experimentally induced chronic model of liver fibrosis in rats associated with clinically relevant co-morbidities, eventually leading to progressive hepatic dysfunction.

MATERIALS AND METHODS

Experimental protocol
Male albino rats of the Wistar strain (200-250 g) obtained from the animal house of our institute were used for the study. Rats were housed in a conventional cage, maintained on a 12 h light-dark cycle and had free access to standard rat chow and water ad libitum. Rats (200-250 g) were divided into six groups, 2 controls and 4 treatment groups, of six rats each. The first group received 0.375 mL/kg olive oil (Loba Chemie Pvt Ltd.) po od ($n = 6$) served as a normal control and carbon tetrachloride CCl$_4$ control group (n

= 6) to which a mixture of CCl$_4$ (Loba Chemie Pvt Ltd.) and olive oil (1:5) 0.375 mL/kg po od thrice a week was administered until the study termination at 7 wk. Silymarin (standard) group (Micro Labs Ltd.) 100 mg/kg po od ($n = $ 6), heparin (test) group (Biological E Limited) 2000 IU/ kg iv od ($n = 6$), silymarin 100 mg/kg po od and heparin 2000 IU/kg iv od treated (combination) group ($n = 6$) was administered for 6 wk (continued with CCl$_4$ injury for 7 wk) and a control treated group received heparin 2000 IU/kg iv od for 6 wk without CCl$_4$ injury.

Collection of blood samples
One day after the last CCl$_4$ administration, blood samples were collected under light anesthesia by the retro-orbital route in anticoagulant free vials. After 30 min, samples were centrifuged at 3500 r/minute for 20 min to separate serum which was used for further biochemical estimations (liver function tests). Fresh venous blood (1.5 mL) was collected in 0.15 mL heparin sodium containing eppendroff. The blood sample was centrifuged at 1500-3000 r/min for 20 min to separate plasma samples which were used to determine the fibrinogen level by the Kjeldahl-Nesslerization method[13]. The rats were sacrificed by an overdose of anesthesia and liver was collected from same set of animals.

Hepatic function measurements (liver function tests)
Terminal measurement of hepatic function was performed by liver function tests in the same animals. Serum glutamate oxaloacetate transaminase (SGOT) (Merck Limited India), serum glutamate pyruvate transaminase (SGPT) (Span diagnostics Ltd.), alkaline phosphatase (ALP) (Span diagnostics Ltd.), total and direct bilirubin (Span diagnostics Ltd.), total protein levels (Beacon diagnostics Pvt Ltd.) were measured using commercially available rat enzyme kits. Indirect bilirubin was calculated by subtracting direct bilirubin from total bilirubin.

A reagent for fibrinogen estimation, stock Nessler's reagent, was prepared by mixing of 150 g of potassium iodide and 200 g mercuric iodide in about 1 L of distilled water, the solution was filtered and final volume was made to 2 L (Solution A). In a separate vessel, 10 g of sodium hydroxide pellets were dissolved in 100 mL of distilled water (solution B). At the time of use, 15 mL of solution A was mixed with 70 mL of solution B and 15 mL of distilled water was added and the solution cooled by refrigeration. Stock standard was prepared by dissolving 0.3772 g ammonium sulphate (Finar Lab reagents) in 100 mL of distilled water to get a 0.3772 g/dL concentration. Working standard was prepared by diluting 5 mL of stock standard solution up to 100 mL using distilled water. This solution contains 4 mg/dL nitrogen. Zero point five mL of plasma was mixed with 14 mL of distilled water and 0.5 mL of calcium chloride solution (2.5 g/dL) (Finar Lab reagents) in a porcelain dish. A fine glass rod was placed in a porcelain dish. The porcelain dish was kept at 37 ℃ overnight so that clot formation was complete. One milliliter of digestion mixture [1 g/dL Selenium dioxide (Loba chemie Pvt Ltd.) in 50% (v/v) sulphuric acid
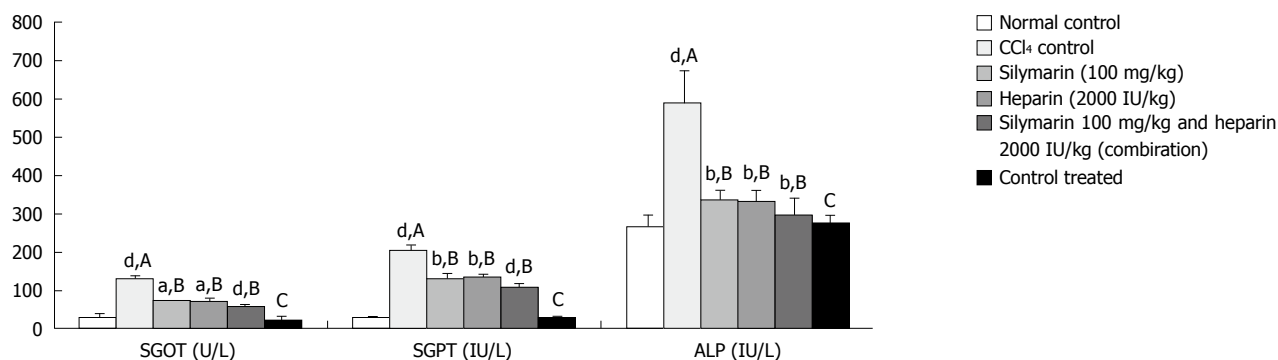
Figure 1 Mean of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase levels measured terminally (*n* = 6). Normal control, carbon tetrachloride (CCl₄) control, silymarin (100 mg/kg), heparin (2000 IU/kg), silymarin and heparin (combination) group and control treated animals. SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase. [a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$. A: Normal control *vs* CCl₄ control; B: CCl₄ control *vs* treatment group; C: Normal control *vs* control treated.

(Thomas baker)] was added to this. The beaker was then heated carefully using a microburner. A drop of 30% w/v H_2O_2 (Rankem Ltd.) was added. The mixture first darkened and with further heating it became colorless. It was then cooled and distilled water was added to make a 10 mL volume and mixed thoroughly. Two mL from this was mixed with 5 mL cold distilled water and 3 mL of cold working Nessler's reagent. After mixing thoroughly, absorbance was taken at 440 nm. For the standard solution, 1 mL of working standard solution was mixed with 6 mL cold distilled water and 3 mL of working Nessler's reagent and absorbance was read at 440 nm. Optical density of the test solution divided by optical density of standard and multiplied by conversion factor 250 gave the concentration of fibrinogen in mg/dL.

Histopathological examination

Liver was collected from the same set of animals at termination for gross and microscopic histopathological examination from all groups. Sections of the liver lobe a few mm thick were taken from each rat and processed for observation by light microscopy. The process involved fixation of the tissue with 4% formalin (Loba Chemie), embedded in paraffin blocks, sectioned with microtome (0.7 U thick) and finally stained by the hematoxylin and eosin stain technique. Hematoxylin stains the nucleus light blue which turns red in the presence of acid. The cell differentiation is achieved by treating the tissue with acid solution and the counter staining is performed by using eosin which imparts a pink color to the cytoplasm. The sections were scanned and analyzed by a pathologist who was blinded to the different treatments in the experiment.

Ethics

This work was carried out in accordance with the principles of the committee for the purpose of control and supervision on experiments on animals and guidelines for animal care were followed.

Statistical analysis

Body weight and liver function test data in text are expressed as mean ± SE of six observations. Differences between any two groups for all data were assessed by one way ANOVA with post test (Turkey). Statistical analysis was performed using a graph pad statistical analysis system. Histopathological changes of varying severity were observed and scored between groups. Percentage area of fibrosis was assessed by *t*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

Effect on liver function

CCl₄ administration (0.375 mL/kg) thrice a week for 7 wk resulted into significant elevation in SGOT, SGPT, aspartate aminotransferase (AST), total, direct and indirect bilirubin levels compared to the normal control. However, no significant elevation was observed in total protein levels. Fibrinogen levels were found significantly decreased after CCl₄ administration compared to the normal control. Heparin, silymarin and heparin-silymarin combination groups showed distinct improvement in liver functional tests compared to the control. Animals treated with heparin, silymarin and heparin-silymarin combination for 6 wk along with CCl₄ administration for 7 wk showed significantly lesser SGOT, SGPT, AST, total, direct and indirect bilirubin levels and slightly higher total protein levels which did not reached a significant level. Heparin, silymarin and heparin-silymarin combination for 6 wk along with CCl₄ administration for 7 wk showed significantly higher fibrinogen levels. The combination of heparin and silymarin was seen to be more effective than the single drug. The heparin treatment group without CCl₄ injury did not show any significant effect on all measured parameters (Figures 1-3 and Table 1).

Histopathological observations

Liver histopathology in the control group showed development of large septa of connective tissue penetrating into the parenchyma with resultant initiation of fibrous septa formation. Progressive septa formation which leads to nodule formation was observed in more severe cases. Treatment with silymarin, heparin and a silymarin and heparin combination reduced the severity of histopatho-

Table 1 Liver parameters measured terminally in normal control (*n* = 6), carbon tetrachloride control (*n* = 6), silymarin (100 mg/kg, *n* = 6), heparin (2000 IU/kg, *n* = 6), silymarin and heparin (combination) (*n* = 6) group and control treated (*n* = 6) animals

Parameters	Normal control	CCl₄ control	Silymarin (100 mg/kg)	Heparin (2000 IU/kg)	Silymarin 100 mg/kg and heparin 2000 IU/kg treated (combination) group	Control treated
Food intake (g/rat per day)	25.87 ± 2.9	24.62 ± 3.5	24.01 ± 2.5	22.56 ± 3.9	23.46 ± 1.5	21.82 ± 4.0
Water intake (mL/d)	145.78 ± 5.1	148.01 ± 6.2	146.71 ± 5.2	140.98 ± 5.5	142.64 ± 7.2	141.72 ± 4.6
SGOT (U/L)	25.4 ± 13.9	124.11 ± 13.4[b]	71.81 ± 3.7[c]	72.32 ± 5.4[c]	56.63 ± 9.2[d]	22.6 ± 11.2
SGPT (IU/L)	27.8 ± 8.3	199.99 ± 15.8[b]	128.17 ± 14.0[d]	132.17 ± 9.4[d]	105.62 ± 12.2[d]	28.6 ± 8.1
ALP (IU/L)	266.6 ± 31.4	583.46 ± 86.1[b]	334.9 ± 24.1[d]	330.86 ± 29.7[d]	296.11 ± 46.1[d]	273.0 ± 25.1
Total bilirubin (mg/dL)	0.96 ± 0.6	8.45 ± 0.5[b]	1.88 ± 0.8[d]	2.30 ± 1.0[d]	1.73 ± 0.3[d]	1.03 ± 0.7
Direct bilirubin (mg/dL)	0.2 ± 0.2	3.34 ± 0.5[b]	0.73 ± 0.1[d]	0.84 ± 0.1[d]	0.38 ± 0.1[d]	0.35 ± 0.3
Indirect bilirubin (mg/dL)	0.85 ± 0.6	5.12 ± 0.8[b]	1.15 ± 0.6[d]	1.46 ± 0.6[d]	1.35 ± 0.2[d]	0.83 ± 0.2
Total protein (gm/dL)	5.8 ± 0.3	5.45 ± 0.2	6.04 ± 0.2	6.22 ± 0.3	6.05 ± 0.2	5.9 ± 0.2
Fibrinogen (mg/dL)	148.89 ± 3.5	38.24 ± 2.3[b]	183.04 ± 16.7[d]	196.99 ± 19.4[d]	246.4 ± 9.2[d,f,h]	149.54 ± 0.3

mean ± SE of 6 observations. [b]*P* < 0.01 *vs* normal control; [c]*P* < 0.05, [d]*P* < 0.01 *vs* carbon tetrachloride (CCl₄) control group; [f]*P* < 0.01 *vs* silymarin and heparin combination group; [h]*P* < 0.01 *vs* silymarin and heparin combination group. SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase.

Table 2 Histopathological scoring of liver sections of control and treated animals

Parameters	Normal control	CCl₄ control	Silymarin (100 mg/kg)	Heparin (2000 IU/kg)	Silymarin 100 mg/kg and heparin 2000 IU/kg treated (combination) group	Control treated
Degree (area) of fibrosis	0	4	3	2	1	0
Percentage area of fibrosis	Fibrosis not observed	58	24.7[dA]	15.7[dA,aB]	12.9[dA,bC]	Fibrosis not observed

Data represented as scores. Scoring was performed as 0 representing normal liver histology (no area/absence of fibrotic area). Scoring 1, 2, 3, 4 represent mild, moderate, high and severe degree (area) of fibrosis respectively. [a]*P* < 0.05; [b]*P* <0.01; [d]*P* < 0.001. A: CCl₄ control vs treatment group; B: Silymarin *vs* heparin 2000 IU/kg treated group; C: Silymarin vs silymarin 100 mg/kg and heparin 2000 IU/kg treated (combination) group.
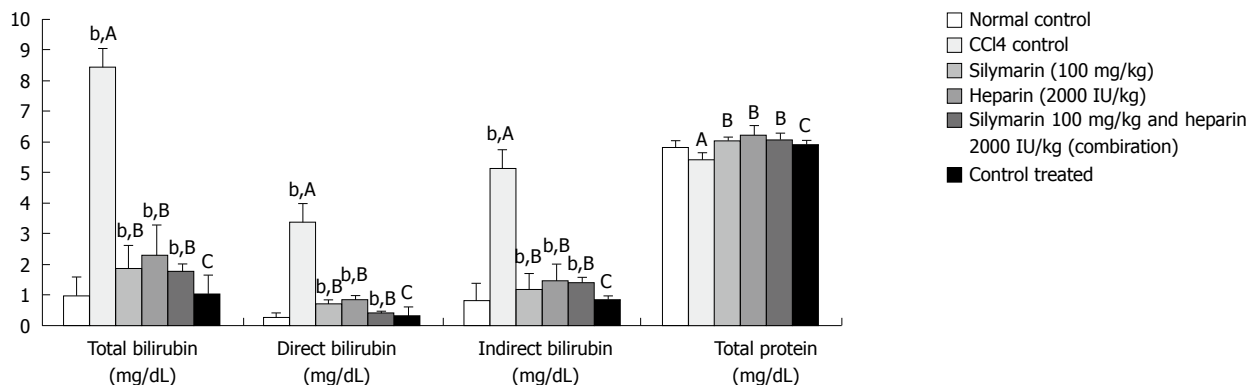

Figure 2 Mean of total, direct, indirect bilirubin and total protein levels measured terminally (*n* = 6). Normal control, carbon tetrachloride (CCl₄) control, Silymarin (100 mg/kg), Heparin (2000 IU/kg), Silymarin and Heparin (combination) group and control treated animals. [b]*P* < 0.001. A: Normal control *vs* CCl₄ control; B: CCl4 control *vs* treatment group; C: Normal control *vs* control treated.

logical lesions compared to the control group. Silymarin, heparin and a silymarin and heparin combination treatment resulted in progressive breakage association with dissolution of fibrous septa and reduction in the area of fibrosis (Figure 4 and Table 2).

DISCUSSION

Liver fibrosis is a disease of chronic liver injury, which is characterized by deposition of ECM[2]. It is one of the ma-

jor causes of death worldwide. All cells in the liver can synthesize and secrete ECM, which regulates the proliferation, differentiation and metabolism of liver cells. The abnormal metabolism and deposition of ECM leads to liver fibrosis. The goal of liver fibrosis therapy is to prevent dysfunction of liver and consequent liver failure. It has been suggested that thrombin plays a detrimental role in chronic liver injury[10]. Fibrin deposition in liver leads to fibrosis[2]. It is well known that thrombin plays an important role in formation of fibrin. Fibrin deposition in vascular beds leads to occlu-
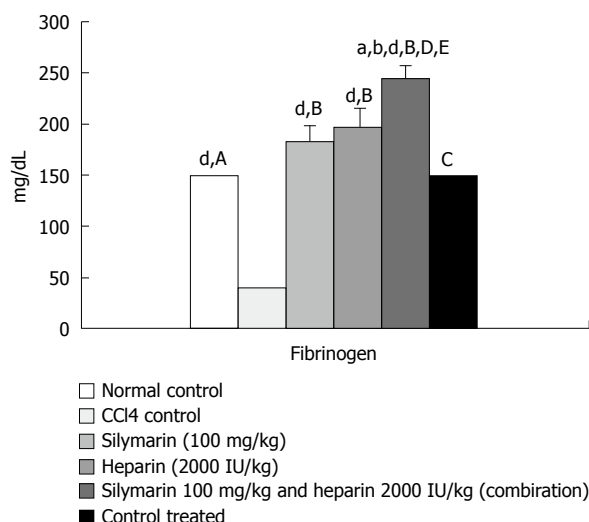
Figure 3 Mean fibrinogen levels measured terminally (*n* = 6). Normal control, carbon tetrachloride (CCl₄) control, silymarin (100 mg/kg), heparin (2000 IU/kg), silymarin and heparin (combination) group and control treated animals. [a]*P* < 0.05, [b]*P* < 0.01, [d]*P* < 0.001. A: Normal control *vs* CCl₄ control; B: CCl₄ control *vs* treatment group; C: Normal control *vs* control treated; D: Silymarin *vs* silymarin and heparin combination group; E: Heparin *vs* silymarin and heparin combination group.

sive thrombi contributing to downstream liver injury.

Thrombin is a multifunctional serine protease that plays an important role in thrombosis and hemostasis. Formed by proteolytic cleavage of prothrombin by the prothrombinase complex, thrombin initiates the final step of the blood coagulation cascade by cleaving soluble fibrinogen to fibrin[14]. There are at least two mechanisms through which thrombin generation could be involved in fibrogenesis. Firstly, clot formation by itself leads to flow disturbance and local hypoxia which is a cofactor for fibrosis[14,15]. Secondly, thrombin signals *via* specific cellular receptors that belong to the family of PAR[15]. The thrombin receptor PAR-1 is up regulated in human liver during acute and chronic injury[6]. It has been reported that thrombin stimulates the proliferation of rat hepatic stellate cells[15]. Chronic thrombin inhibition by synthetic thrombin antagonist SSR182289 reduced the extent of fibrosis deposition in the CCl₄ model of liver fibrosis in the rat[9]. So, it is understood that thrombin plays an important role in liver fibrosis. PAR-1 antagonism protects against experimental liver fibrosis[15]. However, altogether, the case is strong for a role of coagulation proteases in liver fibrogenesis. Heparin is cheap and safe. It has a stronger and direct effect on thrombin. Heparin has been shown to inactivate the coagulation system and prevent acute liver injury[10]. Heparin is reported to produce beneficial effects in patients suffering from liver fibrosis due to chronic hepatitis[12].

We have studied the antifibrotic action of heparin in experimentally induced chronic model of liver fibrosis in rats. Oral administration of CCl₄ is one of the easiest, fastest and reliable techniques to develop fibrosis and can be used to screen antifibrotic agents[9,16-18]. CCl₄ accumulates in the hepatocytes, where its activation by oxidases that are involved in the hemolytic breakage of the C-Cl bond oc-

curs. This activation occurs in the hepatic endoplasmic reticulum *via* an enzyme system of electron transport from reduced nicotinamide adenine dinucleotide phosphate to oxygen, and this leads to formation of a reactive metabolite, the trichloromethyl radical (CCl₃). The CCl₃ radical then binds covalently to a series of molecular structures and particularly to the lipid of endoplasmic reticulum membranes, resulting in the formation of lipid radicals. The lipid radicals react with molecular oxygen to produce peroxy radicals which is involved in initiation of lipid peroxidation[15]. The lipid peroxides produce breakdown of the biomembrane at cellular and subcellular levels. This leads to hepatocyte destruction and release of intracellular enzymes like GOT, GPT and ALP in blood[19,20]. The increased activities of liver marker enzymes such as GOT, GPT and ALP in the serum of CCl₄ induced rats indicate damage to hepatic cells. Both heparin and silymarin treated animals possessed significantly lower SGOT, SGPT and ALP levels compared to untreated animals, suggesting the protective effect of heparin against CCl₄ induced hepatocytes lyses. The much lower levels of SGOT, SGPT and ALP were observed in animals treated with both heparin-silymarin when compared with an individual drug. The combination is more effective in preventing CCl₄ induced hepatotoxicity (Figure 1).

Bilirubin is the excretory end product of heme degradation. It is conjugated in the liver to form bilirubin diglucuronide and excreted in bile. Increase in serum bilirubin levels may be found in hepatocellular damage, hemolytic jaundice and hepatitis[21]. CCl₄ injury causes significant degeneration of hepatocytes and blockage of bile ducts resulted in a significant increase in serum total, direct and indirect bilirubin levels. Heparin and silymarin treated animals had significantly lower total, direct and indirect bilirubin levels compared to untreated animals. The combination of heparin-silymarin shows a much lower level in total, direct and indirect bilirubin levels compared to an individual drug (Figure 2).

The liver participates in synthesis of plasma proteins. Injury to a liver cell causes alteration in liver function by cell necrosis. CCl₄ injury shows decreased total protein levels which was not statistically significant. Heparin, silymarin and heparin-silymarin treated animals had increased total protein levels which is not statistically significant (Figure 2).

Fibrin deposits were in pericentral necrosis areas and within fibrotic septa during liver fibrogenesis, after CCl₄ induced liver damage[2]. During activation of the coagulation system, plasma fibrinogen is converted to fibrin, resulting in a decrease in plasma fibrinogen concentration[10,13]. The decrease in fibrinogen levels is used as a biomarker for activation of the coagulation system and as a monitor of anticoagulant effectiveness. CCl₄ treated animals have shown a significant decrease in fibrinogen levels. Heparin and silymarin treated animals significantly increased fibrinogen levels. However, the heparin- silymarin combination group has shown significant increase in fibrinogen levels (Figure 3).

Histological studies showed that CCl₄ intoxication resulted in development of large septa of connective tissue flowing together, penetrating into parenchyma and
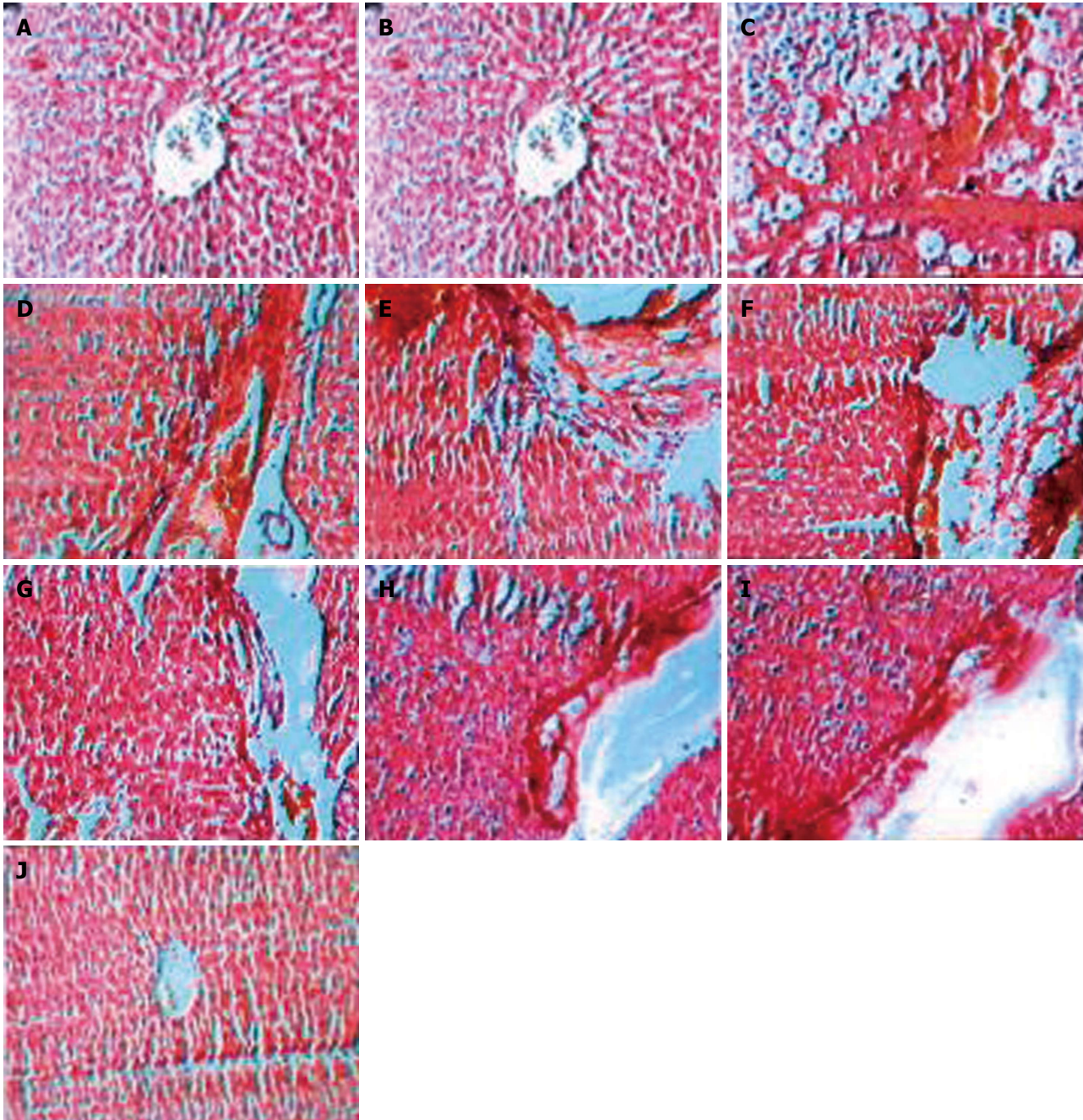
Figure 4 Photomicrograph of liver sections. Normal control (normal arrangement of central vein and intact parenchyma) (A) and carbon tetrachloride control rats development of large septa of connective tissue flowing together and penetrating into the parenchyma, initiation of fibrous septa formation (B) and progressive septa formation which have a tendency to develop nodules of the liver architecture with loss of the structure of the hepatic lobules, seen in (C). Silymarin treated rats showing progressive breakage of fibrous septa (D) and mild degree of fibrolysis with lymphocytic infiltration (E), Heparin treated rats showing progressive breakage of fibrous septa (F) and moderate degree of fibrolysis with lymphocytic infiltration (G), Silymarin and Heparin treated rats showing progressive breakage and almost dissolution of fibrous septa (H) and severe degree of fibrolysis with lymphocytic infiltration (I). Control treated rats showing normal arrangement of central vein and intact parenchymal cells (J).

progressive fibrous septa formation which resulted into nodule formation. Silymarin treated animals showed progressive breakage of fibrous septa with a mild degree of fibrolysis. Heparin treatment resulted in progressive breakage of fibrous septa with a moderate degree of fibrolysis. The heparin-silymarin combination group resulted in progressive breakage and almost dissolution of fibrous septa with a severe degree of fibrolysis.

In conclusion, it can be suggested that heparin pro-

duces significant antifibrotic effects in CCl_4 intoxicated rats. This protective effect could be due to the anticoagulant property of heparin. Heparin binds with antithrombin III and forms the complex which binds with thrombin and blocks its action. Thus, the fibrotic effect of thrombin in liver fibrosis is blocked by heparin, by both blocking the coagulant action of thrombin and also indirectly preventing thrombin to bind to PAR-1 receptors. The heparin-silymarin combination group showed more

of a synergistic effect than either drug alone. However, further studies may be required to support the above conclusion.

COMMENTS

Background
Liver fibrosis is the main complication of chronic liver disease that eventually leads to cirrhosis. Liver fibrosis is characterized by an excessive deposition of extracellular matrix (ECM) components in the liver parenchyma. The treatment of liver fibrosis or cirrhosis is always a problem in clinical practice. To control and stop liver fibrosis towards cirrhosis is of utmost importance. Evidence incriminates the serine proteinase, thrombin, in liver fibrogenesis, either through its procoagulant function or its signaling *via* cell-surface receptors. Recently, thrombin inhibitors have attracted attention as a new class of drugs for the treatment of liver fibrosis.

Research frontiers
Activation of the coagulation cascade classically proceeds through activation of thrombin. There are a few reports suggesting the beneficial effects of thrombin antagonism in liver fibrosis. Many studies reported that direct inhibition of thrombin by a synthetic antagonist decreased lung collagen accumulation in experimental pulmonary fibrosis. Moreover, heparin could inhibit the growth of the Ito cells effect *in vitro*, which suggests that heparin does display some thrombin inhibitory activity.

Innovations and breakthroughs
Heparin has been reported to inactivate the coagulation system and prevent acute liver injury from a hepatotoxic dose of lipopolysaccharide, also effective in cholestatic liver injury in rat. Moreover, the effects of heparin on liver fibrosis in patients with chronic hepatitis B have been reported. There are no clear data for liver fibrosis. The aim of the present study was to evaluate the effect of chronic thrombin inhibition by heparin on experimentally induced chronic liver injury (liver fibrosis) in rats.

Applications
The study results suggest that heparin is a potential therapeutic agent that could be used in preventing liver fibrosis. Additionally, treatment with a combination drug (heparin and silymarin) produced a beneficial protective effect on liver fibrosis.

Terminology
Liver fibrosis: liver fibrosis is characterized by an excessive deposition of ECM components in the liver parenchyma. Thrombin is a multifunctional serine protease that plays an important role in thrombosis and hemostasis. Formed by proteolytic cleavage of prothrombin by the prothrombinase complex, thrombin initiates the final step of the blood coagulation cascade by cleaving soluble fibrinogen to fibrin.

Peer review
This is a good descriptive study in which the authors evaluate the preventive effect of heparin, a thrombin antagonist, on liver fibrosis induced by carbon tetrachloride in rats. The results are interesting and suggest that heparin is a potential therapeutic agent that could be used in preventing liver fibrosis.

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