

# SCREENING MODELS FOR LIVER FIBROSIS

AUTHOR: <sup>1</sup>DHAVANE SHWETA SHAMRAO, <sup>2</sup>AWARE RINKLE ROHIDAS, <sup>3</sup>MUSALE SUMIT KAILAS, <sup>4</sup>NIMBALKAR VIKRAM VINAYAKRAO, <sup>5</sup>GHOLAP SURAJ MACHINDRA.

<sup>1</sup>STUDENT FINAL YEAR M PHARMACY

DR.VITHALRAO VIKHE PATIL FOUNDATION'S COLLEGE OF PHARMACY, VILAD GHAT, AHMEDNAGAR, INDIA, 414111

<sup>2</sup>STUDENT FINAL YEAR M PHARMACY

<sup>3</sup>STUDENT FIRST YEAR M PHARMACY

<sup>4</sup>ASSISSTANT PROFESSOR OF PHARMACOLOGY

<sup>5</sup>STUDENT FIRST YEAR M PHARMACY

DR.VITHALRAO VIKHE PATIL FOUNDATION'S COLLEGE OF PHARMACY, VILAD GHAT, AHMEDNAGAR, INDIA, 414111

## ABSTRACT:

Liver fibrosis basically a process of wound healing in response to various injury, which can leads to liver cirrhosis and can also hepatocellular carcinoma (HCC). The common causes of liver fibrosis are liver injury, alcohol abuse, viral hepatitis B and hepatitis C infection, parasitic infection such as schist soma species, are also responsible in some cases. This paper provides an overview of in vivo and in vitro models used in experimental study of liver fibrosis.

## KEYWORDS:

Liver fibrosis, Experimental models, Extracellular matrix, Hepatic stellate cells.

## INTRODUCTION:

Liver is one of the most important organ which plays vital role in our body<sup>(1,2)</sup>. In body liver works as a site of glycogen storage and detoxifying of harmful chemicals<sup>(3)</sup>. Liver fibrosis is one of the pathological condition which occurs mainly due to continuous injury to liver, including viral hepatitis, alcohol abuse, metabolic disease, cholestasis liver diseases. If liver fibrosis remains untreated it may leads to liver cirrhosis, liver failure and liver cancer which is life threatening or death may occur. For these purpose following are some screening models for liver fibrosis evaluation and treatment<sup>(4, 5, 6.)</sup>.

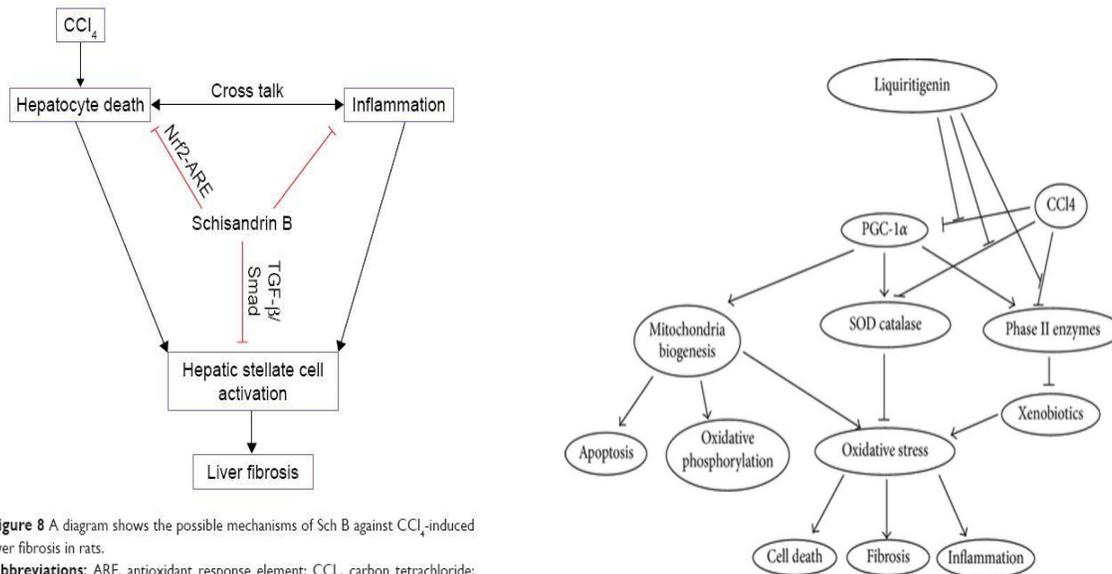
## METHODS

### IN-VIVO MODELS OF LIVER FIBROSIS

#### 1) Chemical based models: -

Various chemicals are known to induce liver fibrosis. In most of cases intraperitoneal injection of these chemicals causes liver fibrosis within short period of time<sup>(8)</sup>. While oral or inhalational administration of drugs may causes liver fibrosis after long period of time<sup>(8)</sup>.

##### a) CCL4 or TAA induced model (pan lobular fibrosis): -



**Figure 8** A diagram shows the possible mechanisms of Sch B against CCl<sub>4</sub>-induced liver fibrosis in rats.

**Abbreviations:** ARE, antioxidant response element; CCl<sub>4</sub>, carbon tetrachloride; Nrf2, nuclear factor-erythroid 2-related factor 2; Sch B, schisandrin B.

Animal (mice) are kept in specific pathogen free condition on a 12 hour light and 12 hour dark cycle. Air conditioned room are maintained at 25 °C and provided with water and standard mouse pellet chow ad libitum<sup>(7)</sup>. Mice receive increasing doses of CCL4 (50/50 volume of mixed CCL4 and mineral oil) three times per week by oral route with help of oral gavage. Dose should not exceed more than 3ml/kg. Solution is stored in room temperature. Or fibrosis can be induced by increasing doses of TAA. TAA is dissolved in 200µl PBS, and given intraperitoneal three times a week as 50 mg/kg for 1<sup>st</sup> and 2<sup>nd</sup> dose in week 1, to 400mg/kg on 16<sup>th</sup> dose onwards after week 6<sup>th</sup>. Fresh solution is prepared every time on every week. Animal (mice) are sacrificed on 1<sup>st</sup> or 2<sup>nd</sup> day after the dosing is completed of CCL4 or TAA. Sacrificed method used is cervical dislocation under suitable dose of general anesthesia. Liver is isolated and weighed. Two lobes of liver are examined and dipped into 4% buffered formalin for further analysis.

## 2) Diet based model: -

These are some typical diet which is used to cause of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis (NASH) in animals under experiments<sup>(9)</sup>. Rodent strain is more determinant of liver fibrosis induced by dietary ingredient<sup>(10)</sup>.

### a) High fat diet: -



High fat (HF) diet overcomes the barrels caused by MCD diet. Since animals leads to gain weight of body and develop insulin resistance. This model requires 50 weeks to develop very mild fibrosis in mice with steatohepatitis<sup>(11,12)</sup>. These models is most suitable to rodents to develop NASH using high fat diet .the model is in contrast to rats as they are not showing response to high fat diet<sup>(13)</sup>. An alternative to this high cholesterol diet has been used in rats. This causes liver fibrosis in 9 weeks in rats, where they develop cirrhosis occasionally<sup>(14)</sup>.

## 3) Surgery based models: -

Common bile duct ligation is known to lead cholestatic injury and periportal biliary fibrosis. Bile duct ligation is a model which is used in rats and mice to induce fibrosis<sup>(21)</sup>. In BDL method is consisting of a doubly ligated bile duct at two point<sup>(22)</sup>. Blocking of bile duct increases in biliary pressure, inflammation and various cytokines secretion by biliary epithelial cells, these leads to increase in expression of fibro-genic markers like α-SMA, TGF

$\beta$ 1, and accumulation of B cells and T cells in portal veins which activates HSCs in fibrogenesis and produces active portal fibroblast in BDL further causes IL13 synthesis<sup>(19, 20, 21, 16)</sup>. This process is reversible upon two weeks after removing obstruction. In general, early mortality in rodents may ensue after BDL due to bile leakage, rupture of biliary cysts or gall bladder<sup>(17, 18)</sup>. The mortality rate is 5-6 weeks after BDL in rats is 20% and mortality rate in mice after BDL is 80% in 10 days. BDL is used particularly in short term studies of liver fibrosis associated with fibrosis<sup>(15,34)</sup>.

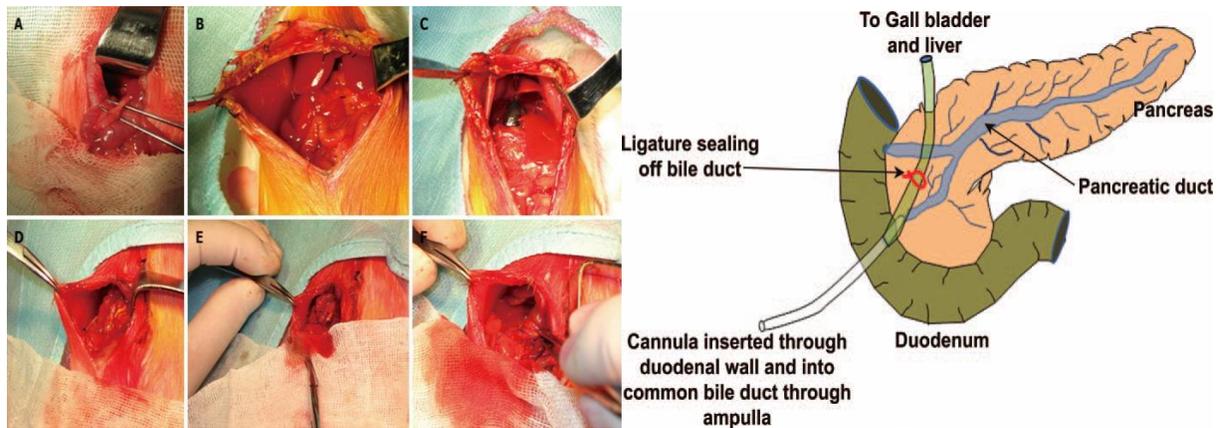


Figure: Bile Duct Ligation

## IN VITRO MODELS OF LIVER FIBROSIS

### 1) Primary hepatic stellate cells: -

Primary HSCs are directly obtained from healthy liver tissue. There are various issues related to original from isolation and cultivation procedures. Classical methods for isolation of HSCs are based on a density gradient centrifuge method using Percoll, Nycodenz, Stractan or Metrizamide. HSCs density is low because of abundant lipid content. Leads to separation from other liver cells type, yielding cell suspension containing up-to 75% of HSC, with a high viability<sup>(31)</sup>. The density gradient centrifugation method cannot be used to isolate HSCs from young animals or animals suffering from liver disease due to low lipid content and poor purity<sup>(32)</sup>. Isolated cells are seeded on a plastic culture dish<sup>(33)</sup>. Freshly isolated HSCs spontaneously activate and turn into my fibroblast like cells as also occurring during liver fibrosis in vivo<sup>(34)</sup>. This spontaneous in vitro activation triggers a differential gene expression profile in comparison with in vivo counterpart process, which may not reflect the path physiological mechanism manifested during liver fibro-genesis<sup>(35, 36, 37, and 38)</sup>.

### 2) Precision cut liver slices: -

Precision cut liver slices (PCLS) is one of the best methods for in vitro study of liver fibrosis. Precision cut liver slices are transfer to the nutrient culture media with a normal thickness of 100-250 $\mu$ m and a diameter of 5mm, which allows nutrient and oxygen to the tissue<sup>(24,25)</sup>. Precision cut liver slices are prepared from healthy and fibrogenic livers can be used for knowing the early or late phases of liver fibrosis<sup>(26, 27)</sup>. Precision cut liver slices are important and interesting to carefully observing various different mechanism involved in occurrence and reversion of liver fibrosis. This method is used for short term purpose<sup>(28, 29, 30)</sup>.

## RESULT

Liver fibrosis is studied in which it shows that liver fibrosis results from a sustained wound healing response to chronic injury, which leads to accumulation of HSCs and collagen on liver leads to fibrosis which further gets converted into cirrhosis leads to liver cancer. The only treatment used primary treatment at fibrosis level or last stage treatment i.e. liver transplantation. Here some models are discussed above which are important in screening for liver fibrosis.

## CONCLUSION

Now a day's liver fibrosis is one of the problem faced by many patient which if remain untreated can causes life threatening condition. Thus there is an urgent need for development and finding of new clinical strategies to manage liver fibrosis. There are some important in vivo and in vitro models which can be used according to convenience of researches, and study design.

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