A Screening Models of (In Vivo And In Vitro) Used for the Study of Hepatoprotective Agents

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Abstract

In order to evaluate hepatoprotective function, both in vitro and in vivo liver or hepatoprotective models have been constructed in the past. These methods examine a drug's potential to prevent or minimize liver damage in test animals. To express their effectiveness and safety in humans, new drugs must first travel through a number of developmental phases, beginning with the identification of their pharmacological characteristics in cellular and animal models. In the medical literature, there are many methods for measuring hepatoprotective efficacy in vivo and in vitro. Fresh hepatocytes are exposed to hepatotoxin treatment in in vitro models, and the effects of the test chemical on those cells are examined. To elicit liver damage in test animals, dangerous dosages of an identified hepatotoxin are administered in in vivo models. The test material is provided before, after, and simultaneously with the toxin treatment. Hepatitis in Long Evans and other chemical agents are commonly employed to generate hepatotoxicity in experimental animals for the evaluation of hepatoprotective medications. Cinnamon rats, liver cirrhosis and necrosis, hepatic fibrosis brought on by carbon tetrachloride in rats, liver cirrhosis brought on by galactosamine, inhibition of proline hydroxylation, trans-epithelial investigations model in dogs, etc. are the various duties that liver cells accomplish. There isn't presently an artificial organ or machine that can accomplish all the tasks of the liver. Some processes are capable of imitating the complete spectrum of liver activities. Liver dialysis, an experimental therapy for liver failure, may replicate certain functions.

Hepatoprotective medications seek to halt or decrease liver damage and boost the liver's capacity to recover itself. These chemicals may be synthetic molecules made via pharmaceutical research or natural compounds derived from plants. They achieve this via a multitude of activities, including liver

1. Introduction

In vitro models are used to treat fresh hepatocytes with hepatotoxins and analyses the effects of the test chemical on those cells. In vivo models use harmful quantities of a recognized hepatotoxin to injure the livers of test animals. Before, after, and simultaneously with the toxin treatment, the test material is delivered. For the study of hepatoprotective drugs, hepatitis in Long Evans and other chemical agents are commonly employed to produce hepatotoxicity in experimental animals. Inhibition of proline hydroxylation, liver cirrhosis in dogs, carbon tetrachloride-induced hepatic fibrosis in cinnamon rats, liver necrosis in rats, caustic chemicals, hepatic cirrhosis in rats, etc. are the various duties that liver cells accomplish. There isn't presently an artificial organ or machine that can accomplish all the tasks of the liver. Some processes are capable of imitating the complete spectrum of liver activities. Liver dialysis, an experimental therapy for liver failure, may replicate certain functions.

Hepatoprotective medications seek to halt or decrease liver damage and boost the liver's capacity to recover itself. These chemicals may be synthetic molecules made via pharmaceutical research or natural compounds derived from plants. They achieve this via a multitude of activities, including liver
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enzyme control, antioxidant activity, anti-inflammatory properties, and encouragement of liver cell regeneration.

A substance's hepatoprotective potential is generally investigated via preclinical and clinical testing. It is normal procedure to test a substance's protective effects on liver health in animal models, such as rats. Toxins or drugs are used to create liver damage in these trials, after which the putative hepatoprotective agent is provided to evaluate whether it can diminish liver damage, reduce inflammation, restore liver function, and stimulate tissue repair.

In the preclinical evaluation of putative hepatoprotective medications, screening models are significant tools. Researchers may use these models to assess the efficacy of novel medicines and better understand the processes behind liver disease. To assess the hepatoprotective impact of natural goods, synthetic compounds, and conventional medications, many screening models have been devised lately. Each model has its own benefits and weaknesses, which helps to offer a full review of hepatoprotective performance.

Models of Hepatoprotective Activity in Vivo

1. Liver disease in Long Evans rats
2. Temporary hepatic ischemia
3. Allyl alcohol caused liver necrosis in rats.
4. Carbon tetrachloride caused liver fibrosis in rats.
5. Rats with bile duct ligation developed liver fibrosis
6. Hepatic necrosis induced by galactosamine
7. Rats Liver damage with Paracetamol.
8. Hepatotoxicity caused by Isoniazid toxicity

Figure 1 In Vivo Hepatoprotective Models

Liver disease in Long Evans rats

It is possible to research genetically transmitted fulminant hepatitis using Long Evans Cinnamon strain rats. There is a connection between this strain of rat and Wilson's disease in humans. Wilson's disease in humans is caused by copper buildup in the liver. A chelation therapy or diet deficient in
copper may be helpful in treating Wilson's disease. This disease and its treatment options can be understood better by studying the Long Evans Cinnamon rat.

**Procedure**

The cinnamon rats are housed in a room with a 12-hour cycle of light and darkness after being acquired from a commercial breeder at the age of five weeks. 6–10 rats are divided into groups of six and ten, and given different diets based on 15% pure egg protein diets with vitamins and drugs supplementary to the diet. Anaesthesia is given by injecting drugs intraperitoneally and administering them through small pumps. Symptoms of jaundice include yellow ears and tails as well as orange urine staining the fur in the abdomen. A week or so after the animal becomes jaundiced, it usually dies. The parameters used in this study are the incidence and mortality of jaundice over time.

**Evaluation**

Stat View II is used for conducting statistical analyses, such as Student's t-tests, ANOVAs, and Scheffé F-tests. Means are used to express all data. The threshold of significance is established at a p-value of 0.05 or below.

**Temporary hepatic ischemia**

Acute liver injuries that result in temporary hepatic ischemia and need to be addressed surgically necessitate liver transplantation. To explore this, a ligature is stretched across the common bile duct, portal vein, and hepatic artery in rats to create complete hepatic ischemia.

**Procedure**

Prior to the experiment, albino guy Holtzman rats weigh 300–350 g are allowed free access to water but fasted for 16 hours. A midline incision is done on anaesthetized rats to access their abdominal cavities. After splenectomy, PE-190 tubes are placed in the right femoral vein and splenic vein to form an extracorporal splenofemoral shunt. Hepatic ischemia is completely achieved by closing the portal vein, hepatic artery, and bile duct. Right femoral artery catheters are inserted to measure blood pressure. The effects of heparin (10 mg/kg) on the liver last for 60 minutes after the administration of heparin.

To replenish lost volume during the ischemia phase, intravenous (i.v.) saline is delivered every 20 minutes. Once the abdominal incision has been closed up, the animals receive either saline (not treated) or medication after the 60-minute ischemia phase is over. The animals are then returned to their original cages after the saline or medicine has been given. Animals used in sham procedures are set up in the same manner, with the difference that no tourniquet is placed on the bile duct, hepatic artery, or portal vein.

**Evaluation**

The \( t \) of indocyanine green clearance for each animal is calculated using a computer software that computes the least squares line of log indocyanine green vs. time. The mean and standard deviation of each group are compared using the student t-test.

**Allyl alcohol caused liver Necrosis in rats**

By using drugs such as antibiotics, it may be possible to avert the focal liver necrosis in rats caused by the injection of allyl alcohol.

**Procedure**

We use 120-150 g Wistar female rats. Water is not denied to the prisoners on the first day, but food is. In order to assess protective effects of compounds, they will either be administered intravenously (IV) or injected subcutaneously (SC) or 3:30 p.m. Orally. A dose of 0.4 ml/kg of allyl alcohol solution in water is administered one hour later. An antibiotic treatment that may be protective is administered at 8:00 a.m. two days later. On the third day, the dog is not allowed to eat or drink. The liver is removed from the animals on the third day at 8:00 a.m.
An inspection of the parietal sides of the liver (lobus caudatus, left, middle, and right lobes) is conducted under a stereomicroscope with a 25-fold magnification. A focal necrosis shows isolated hemorrhagic areas that are white, green, or yellow in color. A necrotic zone's diameter is measured with an ocular micrometre. An index of necrosis is calculated for each animal using these variables.

It is used to examine the parietal sides of the liver (lobus caudatus, left, middle, and right lobes) using a stereomicroscope with a 25-fold magnifying lens. Hemorrhagic foci differ from intact tissue by their color and their conspicuous isolation from white, green, or yellow hemorrhages. Necrotic zones are measured with an ocular micrometre. Necrosis indexes are calculated for each animal based on these variables.

**Evaluation**

The mean necrosis index of ten animals is computed using a student’s t-test, and comparisons between the control and treatment groups are made using that information. Controls are measured as percentage reductions in necrosis indices, while protective effects are expressed in percentage reductions.

**Carbon tetrachloride caused liver fibrosis in rats.**

Chronic carbon tetrachloride injection into rats induces severe hepatic dysfunction and histologically evident liver fibrosis.

**Procedure**

We employ groups of 20 female Wistar rats that initially weigh between 100 and 150 g. Over the course of eight weeks, the animals are administered 1 mg/kg of carbon tetrachloride, dissolved in olive oil 1:1, orally twice a week. The animals are housed in standard circumstances (day/night rhythm, 22 °C room temperature, regular meals such as Altromin 1 321 pellets, and full access to water).

Twenty animals serve as the control group, which only receives olive oil; forty to sixty animals serve as the carbon tetrachloride group, which only receives carbon tetrachloride; and twenty animals serve as the study subject, which is gavaged twice daily to groups of 20 rats based on their body weight (except on weekends, when one dose is given). Animals in eight-week experiments are anaesthetized before being exsanguinated via caval veins.

Total bilirubin, total bile acids, 7S pieces of type IV collagen, and pro-collagen III N-peptides are among the serum constituents that are analyzed. Among the organs that are processed for hydroxyproline analysis are the liver, kidney, aortic wall, and tail tendons.

Organ specimens are weighed before being thoroughly hydrolyzed in 6N HCl. By employing HPLC, hydroxyproline is determined and expressed as mg/kg of the organs' wet weight.

Connective tissue has mechanical properties that can be measured using the following organs: canines, femurs, tibias, and tail tendons. As well as the effect of the on wound wound healing, we investigate how the wound influences healing. Slices of liver about one gram each are used for hepatocellular histology. In each of the three to five sections of liver that are implanted, azocarmine aniline blue (AZAN) dye is implanted. The advancement of fibrosis is then assessed using a score from 0 to IV.

**Grade 0:** Normal histology of the liver  
**Grade I:** Tiny, short connective tissue septa that have little influence on the hepatic lobules' structure.  
**Grade II:** Significant connective tissue septa that enter the parenchyma and flow together. A predisposition for nodules to develop.  
**Grade III:** Loss of hepatic lobule structure and nodular alteration in the architecture of the liver.  
**Grade IV:** Increased production of connective tissues, division of regenerating lobules, and scarring.

**Evaluation**

Statistically significant differences are determined by the unpaired t-test (p 0.05). Comparing the results of histological examinations is done using the 2-test.
Rats with bile duct ligation developed liver fibrosis.

In rats with bile duct ligation, liver fibrosis may be detected histologically and by measuring blood collagen levels.

**Procedure**

Ketanest is used to put male Sprague-Dawley rats under anesthesia. They weigh roughly 250 g. Laparotomies are conducted in sterile surroundings. An incision from the xiphosternum to the pubis reveals the linea alba and the muscle layers. After the skin incision is made, the Linea alba is also incised. An incision is made along the liver border and the duodenum is brought down in order to expose the common bile duct, which runs approximately 3 cm straight from the liver's hilum to the duodenum's entrance. There is no gallbladder; hence, the duct is implanted in the pancreas across the bulk of its length, entering it through a number of smaller channels.

After gently extracting the pancreas, a blunted aneurysm needle is put beneath the section of the duct that was selected, and the duct is then separated using two double ligatures of cotton thread. Cotton stitches are used to seal the muscle layers, the peritoneum, and the skin incision.

**Evaluation**

The unpaired t-test is performed to discover significant differences (p 0.05).

**Hepatic necrosis induced by galactosamine**

D-galactosamine produces acute hepatic necrosis in rats following a single treatment or numerous repeated doses. Cirrhosis is caused by prolonged administration.

**Procedure**

An acute experimental hepatotoxic dose of 0.100 to 0.400 g/kg D-galactosamine is administered intraperitoneally or intravenously to rats over one day. Male Wistar rats weighing 110–180g receive intraperitoneal injections of 500 mg/kg D-galactosamine three times per week for one to three months in order to produce liver cirrhosis. Every day, prospective preventative drugs are taken orally simultaneously with meals. The livers of rats are retrieved by autopsy after they are slaughtered at regular intervals.

**Evaluation**

Light microscopy and immune histology examine livers using antibodies against macrophages, lymphocytes, laminin, fibronectin, desmin, and collagen types I, III, and IV. Immune reactivity is graded from 0 to 4+ for macrophages, lymphocytes, and extracellular matrix components (0 = nonexistent, 1+ = trace, 2+ = mild, 3+ = moderate, and 4+ = severe). GOT and GPT activity are also measured in serum.

**Rats' Liver Damage from Paracetamol**

One of the most popular and commonly used medications is paracetamol. Hepatic damage comes from antipyretic and analgesic medications taken in greater dosages. The cytochrome P-450 enzyme system transforms it into the active metabolite N-acetyl-p-benzoquinone imine, which produces oxidative stress, a drop in liver glutathione, and a decrease in glycogen.

**Procedure**

Wistar rats weighing between 150 and 200g are used. One oral dosage of paracetamol (2g/kg body weight) is administered. The test material is delivered to the animals six days before paracetamol is offered, and on the seventh day, paracetamol is also given. After 24 hours, the animal is slaughtered, the blood and serum are taken for biochemical evaluation, and the liver is used for histology investigation.
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Hepatotoxicity Caused by Isoniazid Toxicity

Procedure

Since 1952, isoniazid (hydrazide of isonicotinic acid) has been used as a first-line antibiotic for TB. It is usually used alone for the prevention of individuals who have normal chest x-ray films and the conversion of their purified protein derivative (PPD), as well as in conjunction with other drugs for the treatment of present illnesses. According to specific studies, slow people belong to slow groups (n = 6 in each category). Ascorbic acid was given to Group I (normal) either before or after intraperitoneal thioacetamide (400 mg/kg b.w.t.) was provided.

With AST, ALT, lactate dehydrogenase (LDH), Y glutamyltransferase, alkaline phosphatase, serum alpha fetoprotein, serum testosterone, and plasma bilirubin and their enzyme activity, we tested the plasma for aspartate amino transferases (AST), alanine amino transferases (ALT), and lactate dehydrogenase (LDH). Treatment with date flesh aqueous extract or ascorbic acid considerably inhibited the rise in plasma bilirubin concentration and enzymes brought on by thioacetamide. Since the initial discovery of its deadly effects, thioacetamide (TA), a potent hepatotoxin, has been intensively explored. It was initially used as a fungicide. A metabolite of TA that links to proteins and produces acetylimidolysine derivatives is thought to cause the hepatotoxic effects of TA.

Rat livers subjected to doses of TA that only produce modest necrosis are stimulated to undergo mitosis and DNA synthesis (B637). The temporal sequence of the increase in DNA synthesis brought on by TA is analogous to that following partial hepatectomy, H-thymidine (H-T) incorporation in treated rats with 50 mg TA/kg body weight shows that these animals exhibit hepatocellular proliferation. which peaks 36 hours after treatment, acetylators are at increased risk of isoniazid hepatotoxicity, indicating that delayed metabolism enables the medication to be metabolised through an alternative route (such as one driven by cytochrome P-450), which may result in the creation of a dangerous metabolite.

Figure-2 Rats' Liver Damage from Paracetamol
Models For Hepatoprotective Activity In Vitro

1. Proline hydroxylation inhibition
2. A canine model for direct transhepatic studies
3. Influence on the synthesis of collagen in human skin fibroblasts
4. Influence on the formation of collagen in Chicken Calvaria
5. Cirrhosis and necrosis of the liver

Figure-4 *In Vitro* Hepatoprotective Models
Proline hydroxylation inhibition

For the collagenous proteins' triple helix's heat stability, the 4-hydroxyproline residues produced by prolyl 4-hydroxylase play a key role in maintaining intramolecular hydrogen bonds. As a result, therapeutic anti-fibrotic medicines may be able to target this enzyme.

Procedure

In a test to determine the level of enzyme activity, 50 milliliters of 50 mM Tri’s buffer (pH 7.5) are diluted with 10-100 milliliters (60,000 d.p.m.) containing 1 milliliter of 2-oxo[1-14C] glutamate, 1-50 milliliters of FeSO4, 0.1-1 milliliter of ascorbate, 10-100 milligrams (Pro-Pro-Gly), 0.1 mg catalase. Thirty minutes of incubation at 37 °C resulted in the formation of 14 CO2 which was trapped and quantified. Rifampin, a drug that is commonly prescribed with isoniazid, raises levels of cytochrome P-450, which supports the idea that the latter drug may be toxic.

Evaluation

A Lineweaver-Burk plot can be used to determine the inhibition modes of a variable substrate (1/v vs 1/concentration plot). Ki values are determined from slopes or intercepts versus inhibitor concentrations in secondary transformation. The least squares approach is used to find the lines of best fit for main plots and secondary transformations. The Ki value has been determined using information from 4-6 studies.

A canine model for direct transhepatic studies

An experimental model of chronic conscious dogs was constructed to perform repeated transhepatic tests over six to eight weeks. This model can help researchers understand how pancreatic hormone synthesis and glucose metabolism affect the liver, and the hepatic pathways connected to high first-pass metabolism and drug interactions with meals, and to research the insulin balance in dogs who have previously undergone islet cell auto-transplantation and pancreatectomy.

Procedure

Two ultrasonic transit time flow probes appropriate for long-term implantation, 4 mm for the hepatic artery and 6 mm for the portal vein, and four silastic catheters, each measuring 0.062 in, a chlorhexidine scrub is used to scrub the ID and OD of the carotid and jugular veins, the hepatic vein of 80 cm, and the portal vein of 70 cm. A 15 cm distance separates the exterior ends of all the devices' velour Dacron cuffs. Sedating and anaesthetizing male dogs weighing 20-25 kg is performed with a 2% halothane/L O2 mixture. Catheters are to be put into subcutaneous pockets and skin interface areas.

Following skin closure, catheters are sealed on the outside. It is then necessary to retrieve catheters and flow probes from subcutaneous compartments and position them in the belly. The portal venous flow probe is placed next, after the insertion of the hepatic artery flow probe. The gastroduodenal artery is constricted to limit extrahepatic blood flow. Then, catheters for the portal vein and hepatic vein are placed. The abdomen is closed after ensuring the catheter is in place. Finally, a jugular venous catheter and a carotid artery catheter are placed.

Evaluation

Carotid artery catheters may be used to obtain blood samples from the right external jugular vein, portal veins, hepatic veins, and carotid arteries. An ultrasound probe is used to measure blood flow in the hepatic artery and portal vein. Carotid artery catheters may be used to obtain blood samples from the right external jugular vein, portal veins, hepatic veins, and carotid arteries. Flow probes in the hepatic artery and hepatic portal vein evaluate blood flow.

Measured values are as follows:
1. The portal vein and hepatic artery are used to transport plasma.
2. Drug concentrations in the right external jugular vein of the liver's hepatic artery, hepatic vein, and portal vein.
In each of the portal vein, hepatic artery, and hepatic vein, these data are used to calculate plasma flow and interval area under the curve.

**Influence on the synthesis of collagen in human skin fibroblasts:**

Prolyl 4-hydroxylase must hydroxylate proline residues in order for fibroblasts and other cells able to synthesize extracellular matrix to produce collagen. The endoplasmic reticulum's cisternae host this enzyme. The endoplasmic reticulum and the cell's outer membrane must thus be crossed by a substance that is meant to inhibit this enzyme. A prodrug that is produced into the active ingredient only in cells of certain tissues, such liver cells, but not in fibroblasts, may be used to target an inhibitor of prolyl 4-hydroxylase to particular organs.

**Procedure**

The 2-aminophenol/penicillin G solution, together with sodium ascorbate 50 mg/ml, 3-aminopropionitrile 60 mg/ml, and 3-aminopropionitrile 50 mg/ml, are incubated with human skin fibroblast cells for 24 hours at 37°C. The potential inhibitor is then administered to the cells for 20 min at different doses, and then 2 mCi [U-14C] proline/ml is added. Five hours of the incubation are spent at 37 °C. The medium is then withdrawn from the cells. Proteins from the medium and cells are hydrolyzed after non-incorporated proline has been removed, and the quantity of hydroxyproline is then determined by amino acid analysis. As a marker for protein synthesis, radioactivity must be thoroughly absorbed.

**Evaluation**

The controls are comprised of six samples, and the inhibitor concentrations consist of two samples each. The fraction of control radioactivity used to test proline incorporation According to the following formula, hydroxyproline synthesis is expressed in proportion to the Hyp/Pro ratio:

\[
\frac{(\text{Hyp/Pro sample}/\text{Hyp / Pro control}) \times 100}{100}
\]

**Influence on the formation of collagen in Chicken Calvaria:**

It is necessary to have pro-pharmaceuticals that quickly penetrate cell membranes and are changed intracellularly to the active agent in order to identify fibrosuppressive medications for therapeutic use. In chicken calvaria, pro-inhibitors that are hydrolytically hydrolyzed may limit collagen production.

**Procedure**

15-day-old chicken embryos have their calvaria removed, and they are then washed in DMEM for three minutes at 37 °C. In the following step, they are placed in Pyrex tubes (each with 8–10 calvaria) with 3 mL medium containing 2 mM glutamine, 6 mCi [U-14C] proline, and a different dosage of inhibitor.

The samples are maintained at 37 °C for 1.5 to 6 hours. By placing the tubes on ice and separating the calvaria from the growth material, the experiment is over. The incubation medium is coupled with a single wash of the calvaria using 3 ml of fresh medium. The final concentrations of phenylmethylsulphonyl fluoride and bovine serum albumin are 1 mg/ml and 6 mg/ml, respectively.

0.5 M acetic acid is used to extract the calvaria for 16 hours in 25 ml. Neither the medium nor the calvaria extracts are affected by the remaining methods. 0.5 M acetic acid is dialyzed completely against the materials at 4°C. SDS-PAGE 1 and triple-helix stability tests are conducted using aliquots. We lyophilize the residual material, resolving it in 6 N HCl, then hydrolyzing it for 24 hours at 105 °C in order to remove the contaminants.

The samples are dissolved in 2 ml of water after the acid has evaporated, and the concentration of hydroxyproline is then determined. Calvaria are incubated for 3 hours under the aforementioned conditions in the presence of 10 mCi [3,4-3H]-proline and 2 mCi [U-14C]-proline in order to evaluate the quantity of collagen hydroxylation and collagen production. Aliquots of medium and calvaria samples are digested after lyophilization.
The link between collagenase-degradable and collagenase-resistant radioactivity is used to quantify the amount of collagen as a percentage of total protein synthesis. The 3H/14C ratio in the digested material is used to evaluate the degree of hydroxylation.

A trypsin/chymotrypsin mixture is used to determine the stability of the isolated collagenous material. The culture media or calvaria extract are incubated at 25 °C to 45 °C in 800 l of 0.04 M NaCl/0.1 Tris, pH 7.4. In an experiment involving 200 liters of a solution of 1 mg of chymotrypsin/ml buffer and 1 mg of trypsin, the sample was digested for 15 minutes at room temperature after being cooled to 0°C. With help from trichloroacetic acid (10% v/v), 100 l of 1 mg BSA/ml buffer are used to precipitate the protease-resistant radioactivity made with triple-helical collagen.

The whole sample is transferred to 2.5-cm diameter Schleicher and Schüll OE-67 filter paper. By repeatedly washing with cold 5% trichloroacetic acid and methanol, the digested material is removed. After drying the filters, the radioactivity is measured. Auto fluorography and SDS-polyacrylamide gel electrophoresis are used to evaluate unhydrolyzed materials. By utilizing electron microscopy, the morphologic properties of the control and treatment cultures are studied.

Evaluation
Concentration response curves are used to visually explain the IC50 values for hydroxyproline synthesis. Proline incorporation is utilized to calculate total protein synthesis, and the mean and standard deviation are determined from four samples.

Cirrhosis and necrosis of the liver
Man-made liver cirrhosis is caused by a number of disorders, including alcoholism, viral hepatitis, intoxications, bile duct issues, inborn illnesses, and others. The gathering of connective tissue and parenchymal regeneration are opposing processes in the development of cirrhosis. Extreme collagen overproduction and connective tissue development in the liver impede hepatic blood flow, affect liver metabolism, and elevate portal vein pressure. Hepatic failure, esophageal hemorrhage, portal hypertension, and other diseases are brought on by these routes.

Therefore, inhibitors of excessive connective tissue formation in the liver are being focused on in the hunt for medications to prevent liver cirrhosis. Collagen is the essential building block of connective tissue, which grows in response to chronic damage. The collagenous fibres' triple-helical building blocks need the creation of hydrogen bonds with the post-translationally hydroxylated amino acid hydroxyproline. If the number of hydrogen bonds is lowered by declining hydroxylated amino acids, the collagen cannot form the triple helix and gets corrupted when deposited in the extracellular matrix. Several hormones, desmotropic drugs, maturation, aging, and desmotropic drugs modify the mechanical activities of connective tissue, such as bone, tendon, and skin.

In addition to minimizing insoluble collagen production in the liver, fibro suppressive drugs also maintain normal collagen synthesis and turnover. A decrease in proline hydroxylation can be used to test for fibrosis-suppressing effects in vitro, but a functional study of connective and supporting tissues along with models of liver cirrhosis and fibrosis will also be required to evaluate the selected organ specificity in vivo.

A chemical or molecule is considered to have hepatoprotective action if it protects the liver from harm or supports its repair. To examine and assess hepatoprotective activity, different models and methodologies are employed. The physiology or pathophysiology of the liver cannot be precisely mimicked by any one model. Hepatoprotective activity is thus generally examined using a mix of diverse models. In addition to giving insights into their mechanisms of action, these models serve as excellent preclinical tools for the assessment of prospective liver medicinal drugs.

4. Conclusion
It is necessary to find novel substances that help in their prevention since liver disorders are a big health concern both domestically and worldwide. The initial phase in the discovery and development of novel pharmaceuticals is the demonstration of their pharmacological characteristics, followed by human safety and effectiveness studies. In vitro models are commonly adopted because they are rapid,
inexpensive, reproducible, and only require a tiny sample. However, different models were used to evaluate the findings. A range of information is offered by the in vivo models. Although they are more luxurious and require more experimental animals, they are commonly used to validate the effects of new compounds. As a consequence, they are often employed as a step before clinical trials, after an in vitro evaluation.

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Ethical approvals

This study does not involve experiments on animals or human subjects.

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