



Exploration of CHIR99021 by GSK-3 β inhibition for triggering the liver regeneration process in partially hepatectomized rats

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ARTICLE HISTORY

Received: 16/02/2024
Accepted: 20/06/2024
Available Online: 05/08/2024

Key words:

Partial hepatectomy, GSK-3 β inhibition, liver regeneration, health, mortality, pharmacy health.

ABSTRACT

The primary treatment for chronic liver disease (CLD) involves dietary and non-dietary interventions. However, when CLD progresses to end-stage liver disease, liver transplantation becomes the only viable option, despite challenges such as donor shortages, transplant rejection, immunosuppressive drug complications, and high costs. To address the urgent need for post-transplantation liver regeneration, the growing focus is emphasized on the stimulation of liver regeneration by pharmacological intervention, even in non-transplanted individuals or those with early liver complications. The objective of this work was to activate the Wnt/ β -catenin signaling pathway by blocking GSK-3 β to enhance the regeneration of hepatocytes and the liver. A partial hepatectomy was performed on 44 rats, with the animals divided into six groups and assessed from postoperative days 1 to 8. The effects of the GSK-3 β inhibitor CHIR99021 (6.25 mg/kg/bw) on hepatocyte regeneration and hepatoprotection were evaluated using biochemical, histopathological methods, and docking studies. It was indicated that both single and repeated doses of CHIR99021 notably improved lipid profiles, liver function, and reduced oxidative stress in partial hepatectomized rats on the third and seventh day as compared to those without drug treatment. Histopathological assessments confirmed substantial hepatocyte regeneration after CHIR99021 treatment, supported by docking studies that showed notable binding interactions with important amino acids of the protein. Mitotic cell phase determination also validated the histopathological findings in different rodent groups. In conclusion, pharmacological stimulation of the Wnt/ β -catenin pathway through GSK-3 β inhibition with CHIR99021 demonstrated promising results in promoting hepatocyte regeneration, potentially offering a new treatment strategy for liver regeneration.

INTRODUCTION

Nearly 2 million people succumb to liver diseases every year, among which complications associated with cirrhosis alone account for 1 million mortalities and the remaining deaths occur due to viral hepatitis and hepatocellular cancer [1]. Almost every organ in the human body has a limited ability to regenerate; however, the liver being an exception, possesses remarkable regeneration capacity after being exposed

to injury or trauma and surgical resection [2]. A vast number of animal models are available for carrying out experimental studies regarding the study of liver regeneration, which can be broadly classified into three categories including toxin-mediated models, surgical models, and progressive hepatic damage models [3]. The partial hepatectomy (PH) can be regarded as one of the pre-eminent surgical models for carrying out liver regeneration studies and their substantial impact can be associated with many forms of hepatocyte injury which play a key role in liver-related disorders [4].

The primary management of chronic liver disease (CLD) involves numerous dietary modifications and pharmacological approaches, including abatement in fat-rich food product consumption and reduction in the use of immune-suppressive and antiviral drugs [5]. However, the CLD can progress to the

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end-stage liver disease, which is characterized by substantial hepatocyte destruction and further accompanied by degradation of the physiological functions. Clinical repercussions in cirrhosis can be attributed to the damage of functional hepatocytes and notable scarring. In such compromised situations, transplantation is the sole alternative for treating severe cirrhosis, but the clinical necessity supersedes the availability of suitable donor organs. Moreover, there are diverse challenges concerned with liver transplantation including rejection of the transplant, donor shortages, complications with long-term use of immunosuppressive medicines, and high cost of the procedure [6]. Thereby, the molecular and cellular mechanisms underlying liver regeneration could provide the path for potential treatment strategies in treating such inimical liver conditions and hence, abolish the need for liver transplantation [7].

The current scenario for treating cirrhosis emphasizes the necessity for rapid initiation of the regeneration process as an alternative to liver transplantation. Notably, in early phases of liver conditions such as cirrhosis, the regeneration process is enhanced by several regenerative pathways via the use of various pharmacological interventions, which can be regarded as potential treatment options.

Several shreds of evidence have reported the initiation of the Wnt/ β catenin signaling pathway via cyclin D1 cascade to be a notable factor for hepatocyte recovery. The TNF- α has an affinity for binding to the receptors on sinusoidal epithelial cells, Kupfer cells, and hepatic stellate cells. Furthermore, this results in the release of the Wnt ligand into the cytoplasm and eventually into the endoplasmic reticulum (ER), where it is activated by the porcupine enzyme [8,9]. The active Wnt protein is considered a crucial stimulator of the typical Wnt/ β -catenin signaling pathway that occurs downstream, which further binds to the frizzled receptor present on hepatocytes and then activates the disheveled protein, which inhibits the activity of GSK-3 [10]. Notably, GSK-3 β , along with other components such as β -catenin, casein kinase 1, and adenomatous polyposis *coli*, is engaged in the production of the destruction complex. The phosphorylation of β -catenin at ser45 occurs via casein kinase 1 and furthermore, the GSK-3 enzyme causes the subsequent phosphorylation of serine/threonine residue. All these events result in the creation of the binding site intended for β -transducin repeat-containing protein, β -propeller, and hence, inducing the proteasome-mediated deprivation of β -catenin. Moreover, the cytoplasmic levels of β -catenin will be reduced in the same pathway, acting as an inhibitory factor for Wnt/ β -catenin signaling [11]. Khurana and Bedi [12] have hypothesized that GSK-3 β inhibition could promote the proliferation of hepatocytes by stimulating the Wnt/ β -catenin signaling pathway, which could further trigger the process of liver regeneration and thereby eliminate the need for liver transplantation and its associated complications.

MATERIALS AND METHODS

Drugs and chemicals

The drugs and chemicals were procured from standard suppliers and were of AR (analytical) grade. CHIR99021 (Purity: >97.0%(HPLC); CAS RN: 252917-06-9; Product

number: C2943) was procured from TCI Chemicals (India) Pvt. Ltd., a division of TOKYO chemical industry and prepared its suspension by dissolving in 0.5% CMC-Na/ saline water (solubility: 5 mg/ml), *N* followed by ultra-sonication.

Animals and ethics statement

The experiments were conducted following the rules established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), India. Inbred male adult Wistar albino rats ($n = 44$) weighing 350–450 g were obtained from an animal house facility, Chitkara College of Pharmacy (Chitkara College of Pharmacy Animal Facility Registration Number:1181/PO/ReBi/S/08/CPCSEA) for the employment of current study with ethical approval number IAEC/CCP/23/01/PR-01. The rats were housed in the departmental animal house in polyacrylic cages at $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (temperature) and humidity (45%–55%) restricted room maintained on a 12-hour light/dark cycle along with the supply of standard rat chow, drink water ad libitum. The experiments were conducted in a semi-soundproof laboratory.

Surgical procedure

A hepatectomy, removing two-thirds of the liver, was performed following the standard approach. Essentially, the technique included administering ketamine anesthesia to the rat, which was positioned in a fully supine stance with all four limbs secured to the surgical table. The liver was revealed by retracting the abdominal wall via a 3 cm midline laparotomy incision. A silk thread with a diameter of 3–0 was looped around the left lateral and median lobes and then pulled up as much as possible toward their starting point. The lobes were then cut off below the tied thread. This treatment involves the removal of around 70% of the whole liver. All rats had the right and caudate lobes of the liver preserved. Following the repair of the abdominal wall, the rats were monitored until they regained complete consciousness, at which point they were permitted to resume oral feeding after around 3 hours. Following all procedures, the animal was given a dose of a prolonged-acting NSAID and 1.0 ml of isotonic saline by injection. Before the surgery and killing, the weight of each rat was measured to determine that there was no notable difference in the average body weight between the sets. The study included six distinct groups for evaluating the pharmacological intervention. These groups were Control, Sham control, Disease control (PH3 and PH7), and Treatment group (PH3 + CHIR99021 at a dosage of 6.25 mg/kg; PH7 + CHIR99021 at a dosage of 6.25 mg/kg). The details of these groups may be seen in Table 1.

Estimation of serum and histological parameters

The rat blood samples were collected through the retro-orbital plexus to measure different biochemical parameters. These included the serum levels of glucose, high-density lipoprotein (HDL) cholesterol, total cholesterol (TC), and triglyceride as well as the liver enzymatic profile. The measurements were performed by means of kits (diagnostic commercial Methods; Erba Mannheim) and an autoanalyzer chem-5 plus V2 semiautomatic. The amount of blood IL-6 was measured using an ELISA kit obtained from Elabscience (USA).

Table 1. Illustration of detailed preclinical experimentation of partial hepatectomized rats and along with other preclinical rodent groups depicting the partial hepatectomized + Drug Treatment (CHIR99021 6.25mg/kg; i.p) work plan.

Normal control group (ncg)							Preclinical group
1ST	2ND	3RD	4TH	5TH	6TH	7TH	Days
						Sacrifice	NCG
Control sham operated group (SH)							
1ST	2ND	3RD	4TH	5TH	6TH	7TH	Days
						Sacrifice	SH
Partial hepatectomized group (PH)							
PH3+DT		Sacrifice					PH3
1ST	2ND	3RD	4TH	5TH	6TH	7TH	Days
						Sacrifice	PH7
Partial hepatectomized group (PH) + drug treatment							
		Sacrifice					PH3+ DRUG
1ST	2ND	3RD	4TH	5TH	6TH	7TH	Days
PH3+DT		PH3+DT		PH3+DT		Sacrifice	PH7+ DRUG

PH third day and seventh day PH3 and PH7; Blue box demonstrating drug treatment (DT); Red box demonstrating the sacrifice and evaluation day.

For the last stage of the experiment, histological examination was conducted using H and E staining.

Assessment of oxidative stress and antioxidant enzyme

The protein content was measured using the modified Lowry method (Standard used: bovine serum albumin), and the reduced glutathione levels were evaluated using Boyne and Ellman's [13] approach. To investigate the thiobarbituric acid reactive substances (TBARSs), the Ohkawa *et al.* [14] method was applied. Cold phosphate buffer saline (pH 7.4; 805 g; 50 mM; 4 C) was used to prepare homogenate of liver tissue for 10 minutes. The top layer of supernatants was then collected for glutathione (GSH) and TBARS measurements [13–15].

In silico studies experimentation

The molecular 3D structures were drawn and refined in the DS4.1 workspace. The current work obtained "X-ray crystal structures of" GSK-3 β complexed with AMP-PNP at a resolution of 2.40 Å from the protein data bank (PDB ID: 1PYX) [16]. The proteins were synthesized to determine the protonation state using the "Prepare Ligands" routine in DS 4.1 at a pH of 7.0 \pm 0.5. Afterward, the resultant compound's molecular geometry was computed. Molecular docking investigations were conducted using the "CDOCKER module of DS 4.1," which is as per the "CHARMm force field" [17]. The "define and edit binding site" tool was used to precisely determine the active site, and the ligand that was synthesized was positioned in the active site of AMP-PNP by docking.

Statistical investigation

The statistical program "Graphpad Prism" was used to compare the statistical differences between several rodent investigational groups using "analysis of variance" ("one-way and two-way ANOVA"). The investigational data was presented as the standard error of the mean (mean \pm S.E.M) and analyzed using *p*-values *p* < 0.05.

RESULT

Effect of GSK-3 β inhibitor (CHIR99021) on body weight in partially hepatectomized rats

It was found that there was no statistically notable difference between all the groups concerned with the body weight on the 0th day, third day, and seventh day (Fig. 1).

Effect of GSK-3 β inhibitor (CHIR99021) on TBARS and reduced glutathione in partially hepatectomized rats

The liver's lipid peroxidation levels were assessed using the liver homogenate technique, which included determining the concentration of TBARS. There was a large (*p* < 0.05) increase in the levels of TBARS in the PH3 group when compared with the control group (Fig. 2A). Additionally, it was noted that the PH7 group exhibited a statistically notable (*p* < 0.05) reduction in TBARS levels compared to the PH3 group, depicting that on postoperative day 7 liver partially healed naturally. Also, the drug-treated group, PH3 + CHIR99021 (6.25 mg/kg) showed a notable (*p* < 0.05) decrease in the levels of TBARS when compared with the PH3. Moreover, the PH7 + CHIR99021 (6.25 mg/kg) treated group showed a notable

($p < 0.05$) decrease in the TBARS levels in comparison with the PH3 and PH7 groups. It was also found that PH7 + CHIR99021 (6.25 mg/kg) group showed notably ($p < 0.05$) dose frequency dependent decreased in the TBARS levels in comparison with PH3 + CHIR99021 (6.25 mg/kg) group.

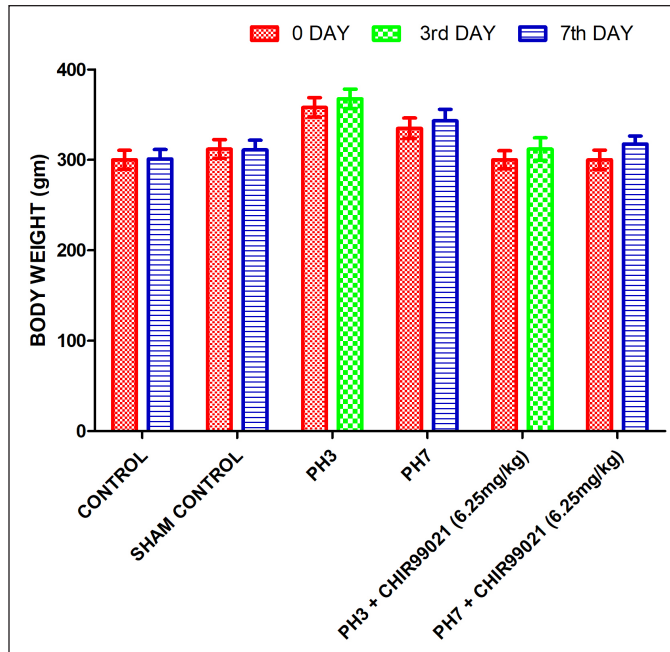


Figure 1. Effect of GSK-3 β inhibitor (CHIR99021) on body weight in partially hepatectomized rats.

It was observed that upon PH, levels of reduced GSH decreased notably ($p < 0.05$) in the PH3 group when compared with the control group (Fig. 2B). On postoperative day 7 (PH7 group), levels of GSH notably ($p < 0.05$) increased when compared with the PH3 group, which signifies that liver partially healed naturally. The PH3 + CHIR99021 (6.25 mg/kg) group showed a notable ($p < 0.05$) increase in the GSH levels when compared with the PH3 group. Also, the PH7 + CHIR99021 (6.25 mg/kg) group showed a notable ($p < 0.05$) increase in the GSH levels when compared with the PH3 and PH7 groups. On comparing both the drug-treated groups, it was found that PH7 + CHIR99021 (6.25 mg/kg) group showed a notable ($p < 0.05$) increase in the GSH levels as of PH3 + CHIR99021 (6.25 mg/kg) group, indicating that increased frequency of dose triggered the liver regeneration more notably.

Effect of GSK-3 β inhibitor (CHIR99021) on liver profile in partially hepatectomized rats

It was found that post PH the levels of total bilirubin (TB), serum glutamic pyruvic transaminase (SGPT), and serum glutamic-oxaloacetic transaminase (SGOT) were considerably increased ($p < 0.05$) in the PH3 set compared to the control set in the mouse model of liver regeneration (Fig. 3A-C). The PH7 group shows a notable decrease ($p < 0.05$) in SGPT, SGOT, and TB levels when compared with the PH3 group which depicted that naturally liver gets partially healed on postoperative day 7. Also, PH3 + CHIR99021 (6.25 mg/kg) treated group notably attenuated ($p < 0.05$) the levels of SGPT, SGOT, and TB when compared with PH3 set (Fig. 3A-C). It was also observed that the PH7 + CHIR99021 group notably lower ($p < 0.05$) the levels of SGPT, SGOT, and TB

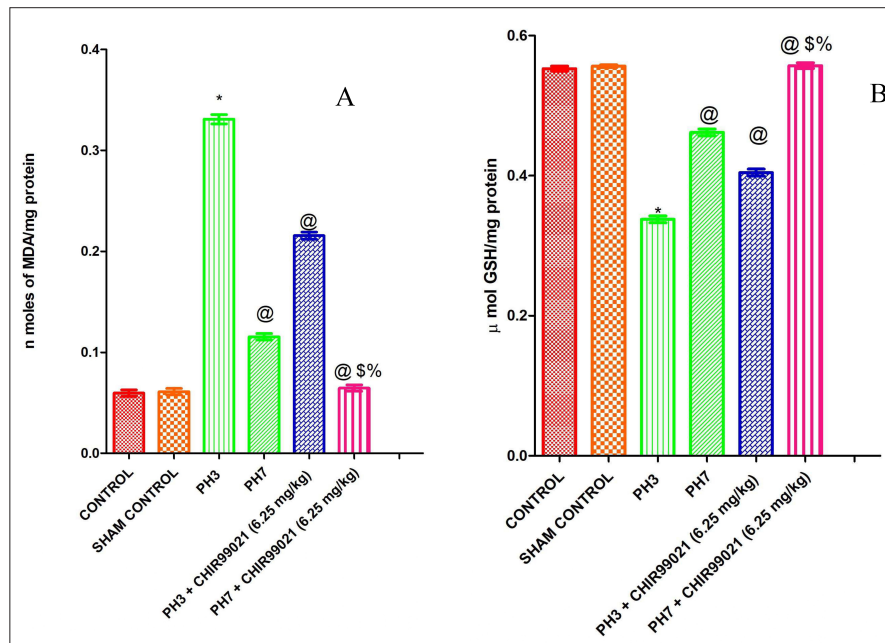


Figure 2. Effect of GSK-3 β inhibitor (CHIR99021) on TBARS and reduced glutathione in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg).

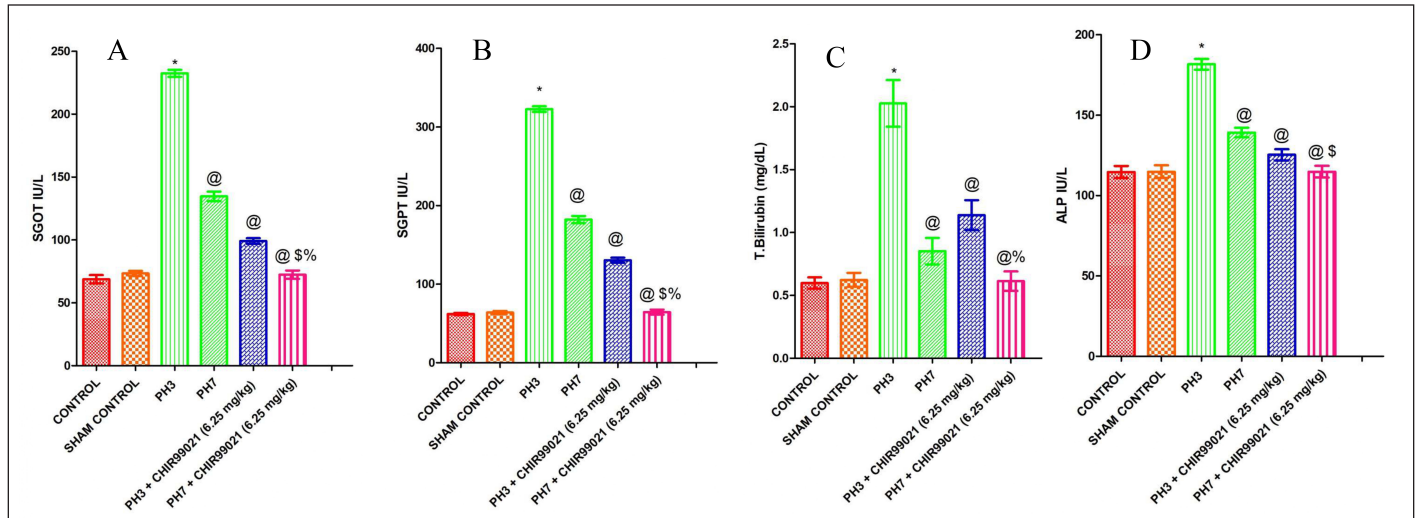


Figure 3. Effect of GSK-3 β inhibitor (CHIR99021) on liver profile in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg).

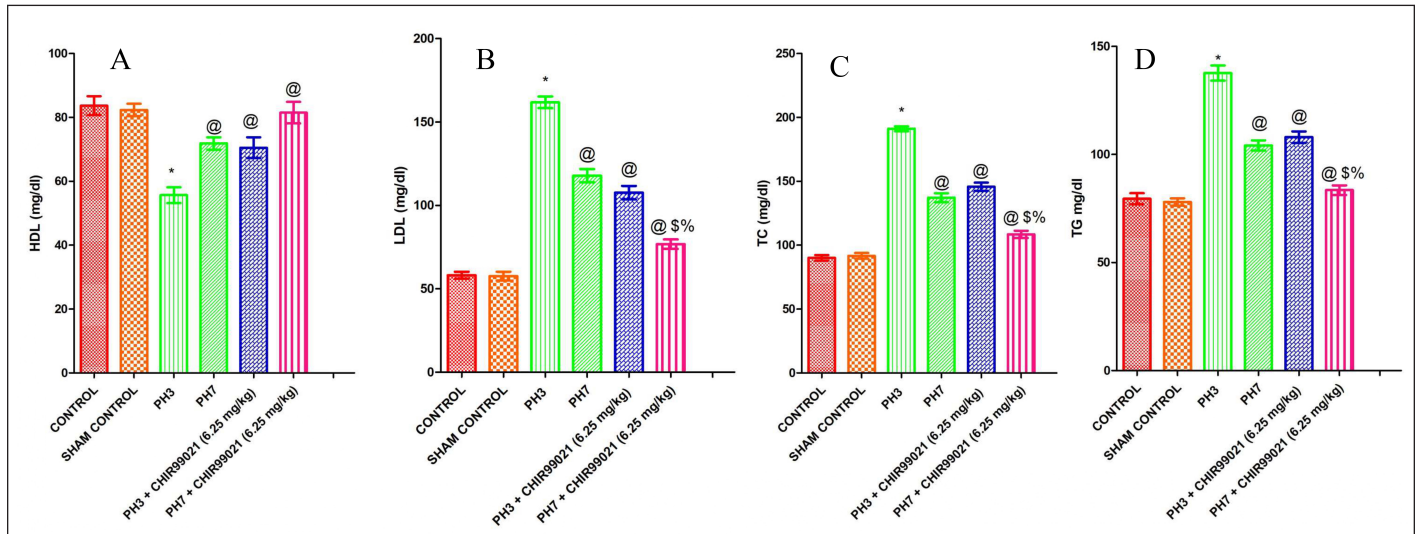


Figure 4. Effect of GSK-3 β inhibitor (CHIR99021) on lipid profile in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg).

when compared with the PH3, PH7, and PH3 + CHIR99021 groups. The PH3 + CHIR99021 (6.25 mg/kg) group shows hepatoprotective consequence but notably less result was noted as compared to dose frequency limits of the PH7 + CHIR99021 (6.25 mg/kg) group. It was also noted that there was a notable decrease in alkaline phosphatase (ALP) levels in PH7 + CHIR99021 (6.25 mg/kg) group as compared to PH7 and PH3 treated groups (Fig. 3D).

Effect of GSK-3 β inhibitor (CHIR99021) on lipid profile in partially hepatectomized rats

In terms of HDL, which is regarded as good cholesterol, there was a notable ($p < 0.05$) decrease in HDL values in the PH3 group as compared to the control group (Fig. 4A). It was observed that the PH7 group has shown notable

($p < 0.05$) increase in the HDL levels when compared to the PH3 group, which depicted that naturally liver gets partially healed. Both the drug-treated groups (PH3 + CHIR99021 and PH7 + CHIR99021) notably ($p < 0.05$) increased the levels of HDL when compared with the PH3 group. Also, PH3 + CHIR99021 (6.25 mg/kg) treated group was non-notable with respect to the HDL levels of PH7 group. PH7 + CHIR99021 (6.25 mg/kg) set showed a notable ($p < 0.05$) increase in the levels of HDL when compared with the PH7 set, possessing CHIR99021 as antihyperlipidemic effect. It was also found that PH7 + CHIR99021 (6.25 mg/kg) group showed notable ($p < 0.05$) dose frequency-dependent increase in HDL levels as compared to the PH3 + CHIR99021 (6.25 mg/kg) set (Fig. 4A). The low-density lipoprotein (LDL), TC and triglycerides (TG) levels were notably ($p < 0.05$) increased in the PH3 group when

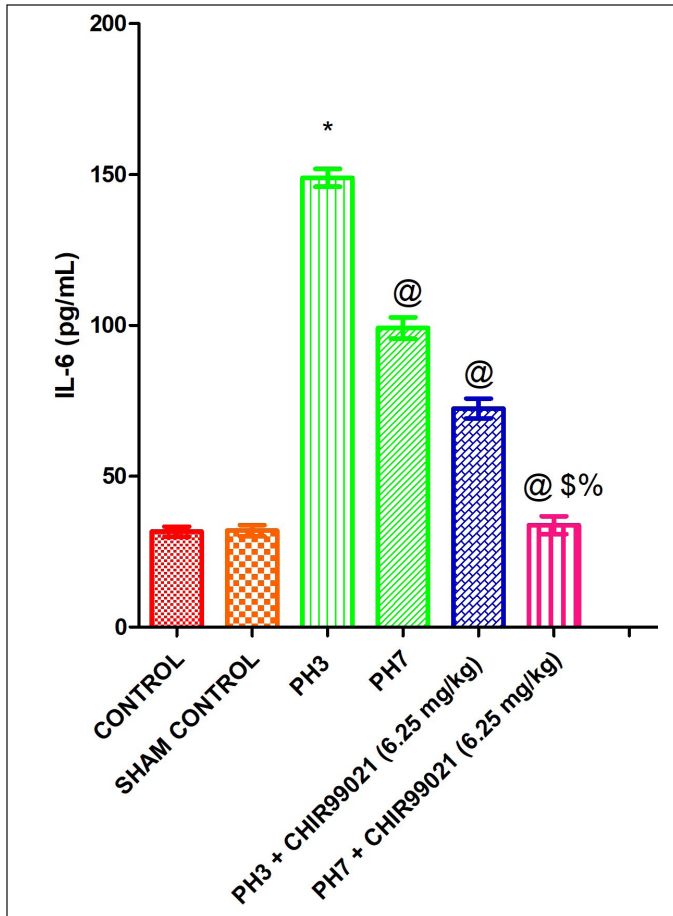


Figure 5. Effect of GSK-3 β inhibitor (CHIR99021) on IL-6 in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg)

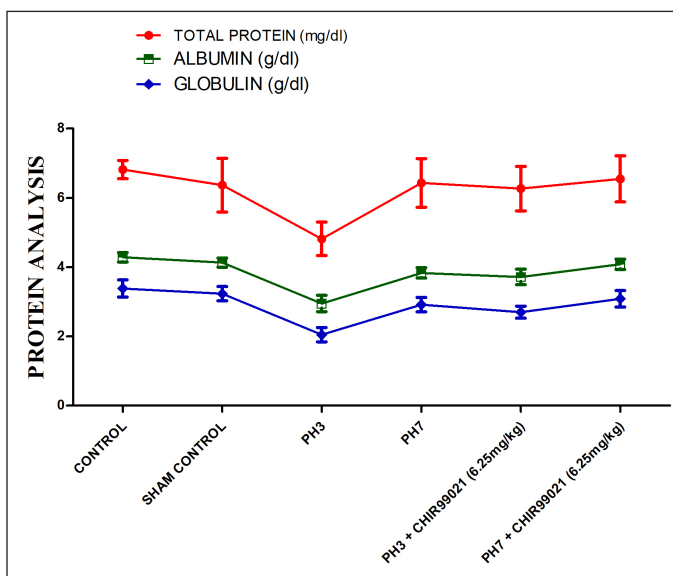


Figure 6. Effect of GSK-3 β inhibitor (CHIR99021) on total protein, albumin, and globulin in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3.

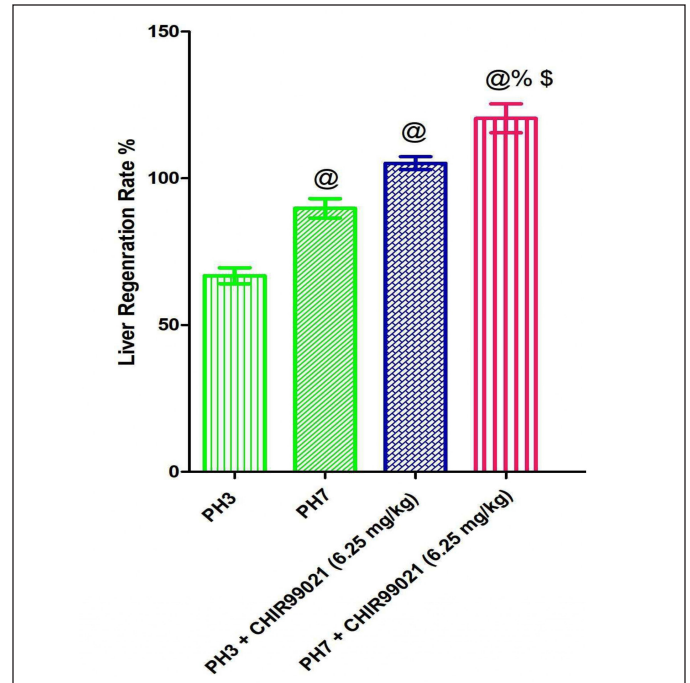


Figure 7. Effect of GSK-3 β inhibitor (CHIR99021) on liver regeneration rate in partially hepatectomized rats.

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg).

compared with the control set (Fig. 4B–D). It was also found that LDL, TC, and TG levels were notably ($p < 0.05$) decreased when compared with the PH7 set. There was a statistically notable drop ($p < 0.05$) in the levels of LDL, TC, and TG in both the drug-treated groups, i.e., PH3 + CHIR99021 (6.25 mg/kg) and PH7 + CHIR99021 (6.25 mg/kg) in comparison to the PH3 and PH7 group. Also, PH7 + CHIR99021 (6.25 mg/kg) group showed a notable ($p < 0.05$) dose frequency-dependent decrease in the levels of LDL, TC, and TG (Fig. 4B–D).

Effect of GSK-3 β inhibitor (CHIR99021) on IL-6 in partially hepatectomized rats

It was observed that IL-6 levels were notably ($p < 0.05$) increased in the PH3 group when compared with the control group (Fig. 5). The PH7 group showed notably ($p < 0.05$) decreased in the IL-6 levels in comparison with the PH3 group. Also, both the drug-treated groups (PH3 + CHIR99021 (6.25 mg/kg) and PH7 + CHIR99021 (6.25 mg/kg) showed notable ($p < 0.05$) reduced in the levels of IL-6 when contrast with the PH3 group. Moreover, on comparison with the PH3 + CHIR99021 (6.25 mg/kg) and PH7 + CHIR99021 (6.25 mg/kg) group, a notable ($p < 0.05$) frequency of dose-dependent decrease in the levels of IL-6 was showed by PH7 + CHIR99021 (6.25 mg/kg) group (Fig. 5).

Effect of GSK-3 β inhibitor (CHIR99021) on globulin, total protein, and albumin in partially hepatectomized rats

A statistically notable reduction ($p < 0.05$) was seen in the range of total protein, albumin, and globulin in the PH3

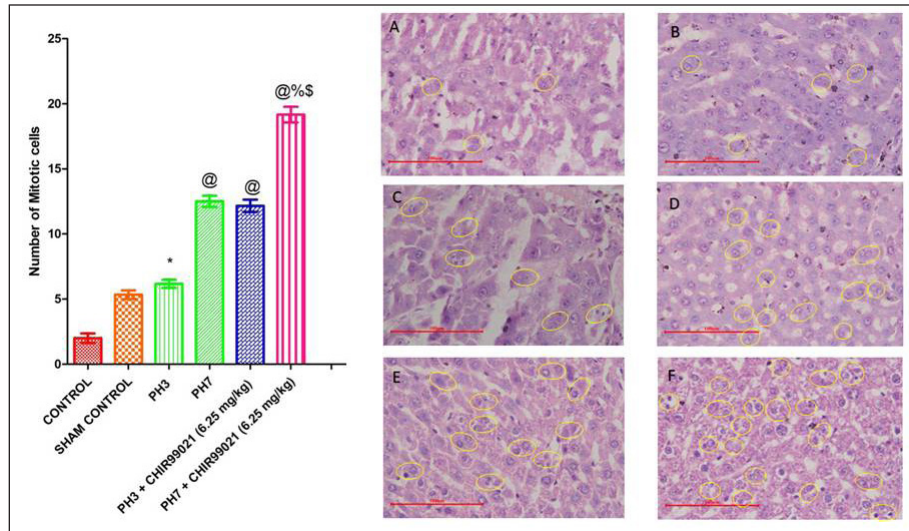


Figure 8. Effect of GSK-3 β inhibitor (CHIR99021) on mitotic index and illustration of number of mitotic cells with reference to the histopathological description in partially hepatectomized rats [Yellow circles depicted the cells in the mitotic cell phase] (Magnification: 500X).

* $p < 0.05$ versus control; @ $p < 0.05$ versus PH3; \$ $p < 0.05$ versus PH7; % $p < 0.05$ versus PH3 + CHIR99021 (6.25 mg/kg).

group in comparison to the normal control set (Fig. 6). It was also noted that levels of the PH7 group were increased notably ($p < 0.05$) when compared with the PH3 group, which signifies that postoperative day 7 naturally liver gets partially healed. Also, PH3 + CHIR99021 (6.25 mg/kg) and PH7 + CHIR99021 (6.25 mg/kg) group showed notably ($p < 0.05$) increased in the globulin, total protein, and albumin levels when compared with the PH3 group (Fig. 6). When both the treated groups were compared, PH7 + CHIR99021 (6.25 mg/kg) group showed notably ($p < 0.05$) increased in the levels of globulin, total protein, and albumin as compared to the PH3 + CHIR99021 (6.25 mg/kg) group, hence, signifies that greater frequency of dose (3 in case of PH7 + CHIR99021 group and 1 in case of PH3 + CHIR99021 group) enhances the serum protein levels and trigger the liver to regenerate fully (Fig. 6).

Effect of GSK-3 β inhibitor (CHIR99021) on liver regeneration rate in partially hepatectomized rats

It was observed that post PH, regeneration rate in the PH7 group was notably ($p < 0.05$) increased when the sets were compared with the PH3 set (Fig. 7A). Also, it was found that both the drug-treated groups [PH3 + CHIR99021 (6.25 mg/kg) and PH7 + CHIR99021 (6.25 mg/kg)] showed notable ($p < 0.05$) augmented in the regeneration rate when contrast with the PH3 and PH7 group, depicting that CHIR99021 treatment accelerates the regeneration rate. Moreover, the PH7 + CHIR99021 (6.25 mg/kg) administered group showed notable ($p < 0.05$) dose frequency-dependent augmentation in the liver regeneration rate in comparison to the PH3 + CHIR99021 (6.25 mg/kg) group (Fig. 7).

Effect of GSK-3 β inhibitor (CHIR99021) on the mitotic index in partially hepatectomized rats

It was observed that microscopic evaluation (Magnification:500X) hepatocytes show regenerative

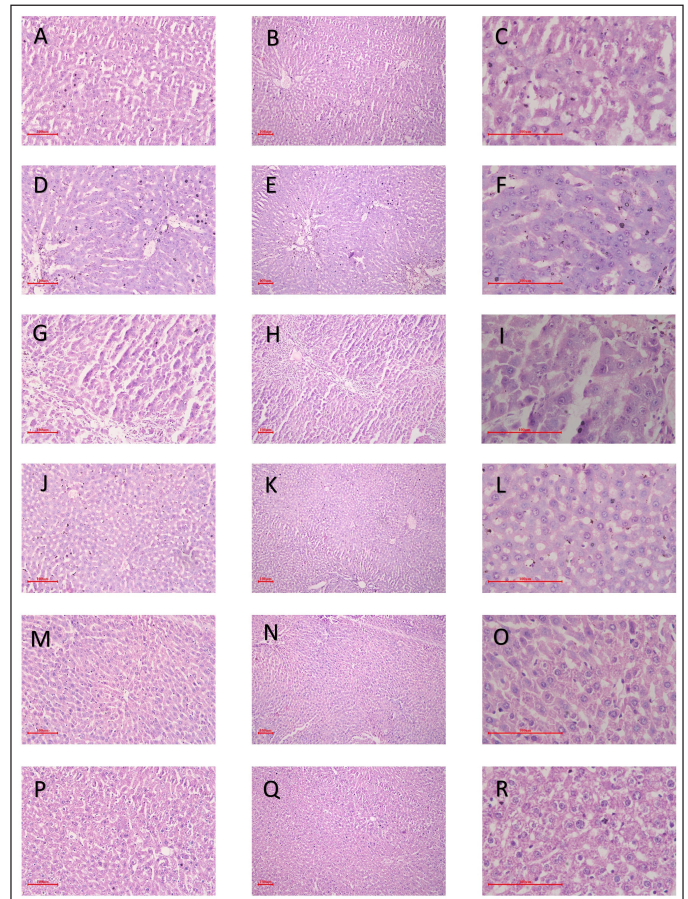


Figure 9. Effect of GSK-3 β inhibitor (CHIR99021) on liver histological changes in partially hepatectomized rats. [A-C: Control Group; D-F: Sham Control Group; G-I: PH3 Group; J-L: PH7 Group; M-O: PH3 + CHIR99021 (6.25 mg/kg); P-R: PH7 + CHIR99021 (6.25 mg/kg)] (Magnification: 100X;200X;500X).

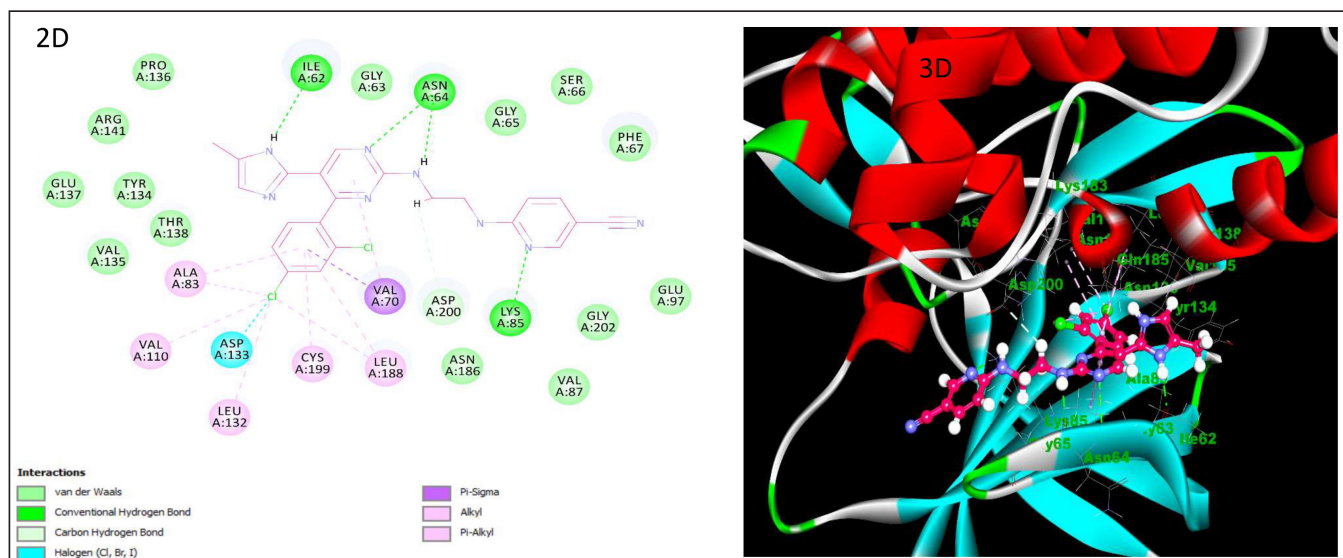


Figure 10. Illustration of docking studies of CHI99021 and their interactions on the active site of GSK-3β (2D and 3D).

appearance in all the experimental groups but with notable differences (Fig. 8). The hepatocyte regeneration was observed with the help of the mitosis phase of the cells which is characterized by higher karyomegali, binuclear regenerative, swollen nucleus (Fig. 8). Mitosis also observed higher number in the periphery and central region. Also, it was found that the PH3 group showed a notable ($p < 0.05$) increase in the mitotic index when compared with the control group (Fig. 8). PH7 + CHIR99021 (6.25 mg/kg) group depicted notably ($p < 0.05$) augmentation in the mitotic index in contrast to the PH3, PH7, and PH3 + CHIR99021 (6.25 mg/kg) group. Moreover, in comparison with both the drug-treated group, it was observed that the PH7 + CHIR99021 (6.25 mg/kg) group showed notable ($p < 0.05$) dose frequency-dependent increase in the mitotic index (Fig. 8).

Effect of GSK-3 β inhibitor (CHIR99021) on liver histological changes in partially hepatectomized rats.

The histopathological results were shown with three different magnifications (100X, 200X, and 500X) to validate the regeneration process of hepatocytes in all the experimental rodent groups. There notable regeneration was found in the hepatocytes in all the groups of partial hepatectomized groups with and without drug treatment as shown in Figure 9 as compared to the normal control group. The liver's hepatic lobule, sinusoidal, plate, and hepatic cell structure was seen to be normal in the PH3 + Drug administered, PH7, and PH7 + Drug administered sets, in comparison to the PH3 partly hepatectomized rats. Furthermore, it was noted that the PH7 + Drug and PH3 + Drug treated groups exhibited a notably higher number of mitotic cells compared to both the normal control group and the PH3-treated rats group, as seen in Figure 8. Based on the aforementioned results, it was seen that there was notable regeneration in the PH3 + Drug administered; PH7, and PH7 + Drug administered groups in contrast to the PH3 partly hepatectomized rats.

Illustration of docking studies of CHI99021 and their interactions on the active site of GSK-3β

Docking experiments were performed on GSK-3β using the “CDOCKER module of DS4.1,” which is as per the CHARMm force field. The findings demonstrated notable docking connections between the compounds and the amino acid residues of the enzyme. When CHI99021 was docked into the enzyme's active site, it notably interacted with the important residues of amino acid along their active site area. The docking interactions of CHI99021 are shown in Figure 10 A and B, using 2D and 3D schematics, respectively. CHI99021 demonstrated substantial aromatic π - π stacking and hydrogen bonding interactions with the crucial amino acid residues located in the active area of GSK-3β. This indicates a beneficial location of the ligand inside the pocket of the GSK 3β enzyme where binding is observed. The primary interactions of significance are conventional hydrogen bonding, hydrophobic interactions (alkyl and π -alkyl), and halogen interactions (chlorine). Compound CHI99021 was resolved by the formation of hydrogen bonds with Ile62, Asn64, and Lys85, halogen bond with Asp133, and finally, hydrophobic interactions with Ala83, Val110, Leu132, Leu188, and Val70. According to the aforementioned findings, essential amino acids and hydrogen bonds both contribute notably to the stability of the ligand-receptor complexes.

DISCUSSION

Liver failure is a rare and progressive clinical condition that is characterized by the rapid loss of functional viability of hepatocytes [18]. According to etiological evidence, liver failure is caused by various disorders, including liver cirrhosis, fibrosis, hepatitis virus (viral infection), and hepatocellular adenocarcinoma, which at the end stage leads to chronic liver injury. After partial liver resection, remaining quiescent liver cells are capable of entering the mitotic phase of cell division. As per research, each leftover hepatocyte has the potential to divide at least 1.6 times to recover the original

liver mass fully [19]. Subjects suffering from liver failure still have the chance for the liver to regenerate, and if we trigger this regeneration process by intervening in the molecular signaling via specific targeted molecular agents, we can easily manage the patient's health. It was demonstrated that there was a pathological change in the levels of various biochemical markers such as liver profile (SGPT, SGOT, ALP, TB, total protein, albumin, and globulin), cholesterol profile (CH, LDL, VLDL, HDL, and TG), and oxidative stress reported in partially hepatectomized rodents as well as in the liver compromised subjects and similar results were also observed in our investigation [20].

As per the literature survey, it was proposed that treatment with CHIR99021, a GSK-3 β inhibitor after 1 hour and with consecutive intervals of PH, leads to elevated levels of β -catenin which is involved in the Wnt signaling cascade for triggering the liver regeneration process. The previous studies reported that CHIR99021 is involved in the self-renewal and differentiation of stem cells/ progenitor cells such as embryonic stem cells, and mesenchymal stem cells [12,20,21]. Moreover, CHIR99021 is also involved in the proliferation and regeneration of various cells such as non-small-cell lung cancer cell lines [22], cardio myocytes [23], neuronal progenitor cells [24], bone tissues, hair follicle formation by stimulating human dermal papilla spheroids [25], alveolar epithelial cells and promote lungs regeneration, human corneal endothelial cell proliferation and promotes feather follicle growth and development [26]. It was also demonstrated that the aid of the CHIR99021 drug along with the cocktail of other molecules enhances the differentiation of bipotent progenitor cells into matured hepatocytes and functionally biliary epithelial cells [27]. From this, we get a lead to choose this drug to trigger the Wnt- β catenin pathway for accelerating the liver regeneration process. However, to the best of our knowledge, no *in vivo* work has been done with respect to liver regeneration via stimulating this signaling cascade in particularly liver-compromised subjects. According to earlier research evidence, it was revealed that there was notable restoration of the liver after 7 days of PH in rodents without the aid of pharmacological intervention [28]. Several reports are there that showed the stimulation of the liver regeneration process other than the Wnt- β catenin pathway in the PH rodents. Therefore, in the current study, the novel signaling pathway was explored to validate the regeneration potential of selected pharmacological intervention.

The current research assessed the considerable reduction in liver function test levels by the CHIR99021 medication (SGOT, SGPT, and ALP) and lipid profile (CH, TG, VLDL, HDL, and LDL) in comparison to the partially hepatectomized group. Similarly, Ohana *et al.* [29] reported that CF102-treated rats post PH decreased the SGOT and SGPT levels, which are crucial markers for the determination of liver failure. Recently, results related to the decreased lipid profile levels after 70% PH were reported in studies conducted by Jo *et al.*, 2020, as well as similar results were found in present studies. In the present study, the oxidative stress levels were also notably increased in PH rats as compared to the drug-treated rodent groups. There was a notable improvement in the antioxidant levels in the PH + drug-treated rats as compared to the PH rodents group alone.

Molecular docking studies play an important role in the drug design process by predicting the binding location of ligands to their active sites and determining the binding affinity of ligands [30]. GSK-3 β is a regulatory serine/threonine kinase that has numerous biological substrates. The GSK-3 β protein is comprised of well-defined three nucleotide phosphate groups and two magnesium (mg²⁺) ions when complexed with AMP-PNP as per their crystal structure. The hydrophobic region of this protein comprised of some important residues Ala83, Val70, Leu132, Val110, Ile62, Tyr134, and Leu188, whereas the hydrogen bonding region consists of some crucial amino acids Asp113, Val135, Glu185, and Arg145 [31]. Therefore, this enzyme is capable of accommodating a variety of different molecular scaffolds. To examine the preferred way in which the test ligand CHIR99021 binds to the enzyme GSK-3 β , docking research was conducted using the given details.

CONCLUSION

In conclusion, it was concluded that GSK-3 β inhibition leads to the progression of the liver regeneration process in partially hepatectomized rats. The selected pharmacological intervention, i.e., CHIR99021 showed a notable hepatoprotective and liver regeneration effect by normalizing the level of liver profile enzymes and by improving the regeneration rate. Moreover, there is an unmet need to validate the *in vivo* results through different *in vitro* and *in silico* studies which is yet to be explored with the help of advanced techniques and biochemical parameters. However, the present evidences give a good insight into CHIR99021 as a better option for preclinical liver regeneration studies for the management of liver cirrhosis and fibrosis.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Chitkara University administration for their continued support in the successful completion of this manuscript.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The experiments were conducted following the guidelines issued by the Committee for Control and Supervision

of Experiments on Animals (CCSEA), India. The protocol of this study was approved by IAEC with approval number IAEC/CCP/23/01/PR-01. Inbred male adult Wistar albino rats (n = 44) weighing 350–450 g were obtained from an animal house facility, Chitkara College of Pharmacy (Chitkara College of Pharmacy Animal Facility Registration Number: 1181/PO/ReBi/S/08/CPCSEA).

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:

Khurana C, Sapra V, Silakari P, Panesar G, Chaudhary M, Gurjeet Singh T, Bedi O. Exploration of CHIR99021 by GSK-3 β inhibition for triggering the liver regeneration process in partially hepatectomized rats. *J Appl Pharm Sci.* 2024;14(08):206–215.