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An Inhibitory Effect of Bone Marrow-Mesenchymal Stem Cells and Hydrogen Sulphide on Liver Fibrosis via the Transforming Growth Factor β signaling

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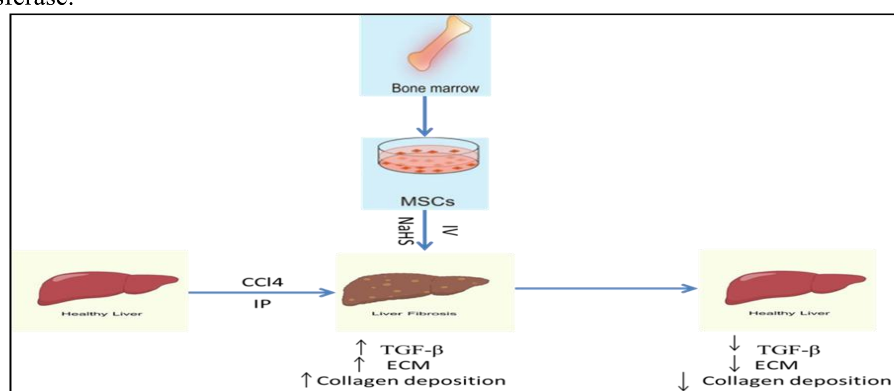
Abstract: One degenerative lesion brought on by a number of chronic liver illnesses is liver fibrosis, which can be efficiently treated by bone marrow mesenchymal stem cells (BMSCs). The goal of this investigation is to assess the potential value of preconditioning BMSCs with sodium hydrogen sulphide (NaHS) as a way to improve the effectiveness of stem cell treatment in rats with CCl4-induced liver fibrosis. Subjects and Methods: Fifty white albinos rats were divided equally into 5 groups (10 rats each); the 1st group served as a normal control, the 2nd group was a fibrosis control, in which rats received 2 mL/kg CCl4 (1:1 corn oil), the remaining three groups received, in addition to CCL4, a NaHS solution (10 mmol/kg), MSC (3x106 cells per rat), and MSCs pretreated with H2S. At the end of the experimental period the following parameters were measured: hepatic transforming growth factor β1 (TGF-β1), which is a specific fibrosis biomarker; serum alanine transaminase ALT and albumin, as liver function biomarkers; and liver histopathological study. Results: The biochemical parameters indicated above were greatly improved by MSCs pretreated with H2S, and the liver sections produced from this group demonstrated a notable improvement in histopathology. Conclusion: The study demonstrated how NaHS pretreatment might improve the effectiveness of MSC therapy in rats with CCl4-induced liver fibrosis.

Keywords: Hepatic fibrosis; Oxidative stress; Reactive oxygen species; Carbon tetra chloride; Alanine aminotransferase.



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1. INTRODUCTION

Liver fibrosis is one of the most serious health issues in the world, which results in inflammation, tissue remodeling, healing processes, apoptosis or necrosis, and apoptosis or necrosis. A significant contributor to hepatic fibrosis is excessive

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extracellular matrix (ECM) synthesis, particularly type I & III collagen [1, 2].

Oxidative stress, which is a typical process of hepatic damage resulting in hepatic fibrosis, cirrhosis, infectious hepatitis, hepatic cell carcinoma (HCC), and other conditions, is brought on by reactive oxygen species (ROS) [3]. Oxidative stress is frequently produced using the xenobiotic carbon tetrachloride (CCl4). It causes lipid peroxidation that is mediated by free radicals, which leads to an accumulation of oxidation products formed from lipids that cause liver injury and excessive liver collagen buildup in the liver, which causes liver fibrosis. Additionally, it aids in the activation of HSCs, the primary collagen-producing cells in the liver [4, 5].

Early detection of chronic liver disorders (CLD) permits the implementation of particular interventions or therapies to halt disease development and increase survival [6]. The principal treatment for end-stage hepatic fibrosis is a liver transplant. The lack of volunteers, difficult operation, transplant rejection, and expensive prices are only a few of its many drawbacks. A newly developed innovative strategy for managing patients who have hepatic fibrosis is MSC therapy [7].

MSCs have been reported to be a successful treatment for liver injury due to their simplicity in isolation, good in vitro proliferation, low immunogenicity, and lack of ethical concerns. MSCs can go to damaged sites and have impacts on differentiation or anti-inflammatory effects [8]. BMSCs possess the ability to differentiate into hepatocytes, myocytes, chondrocytes, osteocytes, and adipocytes in the mesoderm, as well as into ectodermal cell lineages that include neurons and chondrocytes. Pluripotency markers have been detected in BMSCs, indicating that they have the potential to develop into all three germ layer cell lineages [7]. BMSCs may ameliorate hepatic fibrosis and enhance the function of the liver by differentiating into hepatocytes, providing immunological control, producing cytokines and other nutritive elements, decreasing hepatocyte death, and stimulating hepatocyte rejuvenation [9].

Over the past 20 years, hydrogen sulphide (H2S) has gained recognition as an intrinsic modulator of a variety of biological processes [10]. H2S is classified as a gasotransmitter together with nitric oxide (NO) and carbon monoxide (CO) [11]. Enzymes that control the generation of H2S in mammalian cells include cystathionine beta synthase (CBS), cystathionine gamma lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [12]. H2S has several known positive effects, including the ability to protect cells against reactive oxygen species both directly (by functioning as a scavenging agent) and inadvertently (by encouraging the cell's production of antioxidant proteins) [13]. Numerous investigations have shown that the administration of sodium hydrogen sulphide (NaHS), a supplier of H2S, alleviates acute hepatotoxicity caused by CCl4 as well as liver injury brought on by hepatic ischemia-reperfusion, cell apoptosis, and histological alterations [14].

Subtle modifications to stem cell therapy have enhanced the hepatic performance of CCl4-induced hepatic fibrosis. Making the appropriate changes is essential to boost stem cell therapy's efficacy and success rates [15]. It is preferable to use H2S and BMSCs together rather than either of them alone to treat liver fibrosis brought on by bile duct ligation [14]. The current study set out to investigate the effects of the hydrogen sulfide donor NaHS on the effectiveness of MSC therapy in rats with CCl4-induced liver fibrosis.

2. METHODS

2.1. Animals

50 female adult albino rats of the same age and weight (6 weeks old and 120-150 g), of the inbred strain (Cux1: HEL1). The Faculty of Medicine at Cairo University only used inbred animals for experimental purposes. Rats were kept following established protocols and with the approval of the institutional animal handling and utilization committee (NIH Publication No. 85-23, revised 1996). The animals were given a week to adjust to a 12/12 h light/dark cycle, 22 °C, and 2 °C before being employed in the experiment. Rats should be kept in a temperature range of 64°F to 79°F (18°C to 26°C) with 30% to 70% humidity. They were also kept in standardized environmental circumstances, fed a standard feed, and provided a standard diet.

2.2. Chemicals

Each ingredient used in this manuscript was bought from Modified Eagle's Medium (MEM) by Dulbecco (GIBCO/BRL) and is of pure analytical grade. Additionally, sodium hydrogen sulphide (NaHS) was bought from Sigma in the US (Sigma-Aldrich, St. Louis, MO) for use as an exogenous hydrogen sulphide (H2S) donor. Other substances appeared to have an analytical quality.

2.3. Liver fibrosis induction

To cause liver fibrosis in the rats, CCl4 was administered intraperitoneally (I.P.) twice weekly for five weeks with a dose of 2 mL/kg CCl4 (1:1 corn oil) (Sigma, St. Louis, USA) [16].

2.4. Preparation and identification of BMSCs

The bone marrow was removed from male Wistar rats weighing 100–120 g by flushing them with 10% foetal bovine media added to Dulbecco's adapted Eagle's media (Life Technologies, Gibco BRL, USA). Using density gradient centrifugation, nucleated cells were separated via Ficoll-Paque (Pharmacia Biotech, Sweden), and 1% penicillin/streptomycin full growth medium (Life Technologies, Gibco BRL, USA) was then used to suspend the cells again. As a primary culture, cells were kept for 12–14 days in 5% humidified CO2 at 37 °C. When valuable colonies had developed, the cultures were twice rinsed using phosphate-buffered saline (PBS), and the cells were then trypsinized for five minutes at 37 °C using trypsin (0.25 percent) in 1 mM EDTA. Centrifuging the cells at 200 x g for five minutes before re-suspending them in a medium containing serum [17]. Under a light microscope, BMSCs in culture were distinguished from other cells by their plastic stickiness and spindle-shaped fibroblast-like morphology [18]. MSCs' capability to develop into adipocytes, osteocytes, and chondrocytes was demonstrated by their capacity to do so. The red-colored fluorochrome PKH26 (Sigma-Aldrich Co., USA) was used to mark isolated BMSCs [19]. So that their homing may be discovered later. Approximately 1×10^7 BMSCs were collected and suspended again in a small amount of media free of serum for each implantation [20].

2.5. Basic experimental design

Five groups of ten rats each were created from a pool of fifty rats at random:

Group 1 (G1) (normal control): A healthy control group of ten rats was employed.
Group 2 (G2) (CCL4): To cause liver fibrosis in the rats of this group, CCl4 was administered intraperitoneally (I.P.) twice weekly for five weeks with a dose of 2 mL/kg CCl4 (1:1 corn oil) (Sigma, St. Louis, USA) [16].
Group 3 (G3) (CCL4 + H2S): Every two days for a period of six weeks, 1 ml of a NaHS solution (10 mmol/kg body weight) was intraperitoneally injected into LF rats [21].
Group 4 (G4) (CCL4 + MSCs): LF rats were given one dose of MSC ($3x10^6$ cells per rat) intravenously [22].
Group 5 (G5) (CCL4+H2S+ MSCs): After preconditioning MSCs in culture with 200 μmol/L NaHS for 24 hours, LF rats received a single dosage of $3x10^6$ MSC cells per rat [23].
Next, 24 hours after the last treatment, blood was taken from the retro-orbital vein. Animals were then sacrificed via carbon dioxide narcosis, and hepatic tissue was removed for examination. After 15 minutes of waiting for the blood to coagulate, samples of fasting blood were spun up at 3000 g for fifteen minutes for serum extraction. For biochemical analysis, sera were maintained at -80 °C.

2.6. Extraction of mRNA and real-time polymerase chain reaction (RT-PCR)

Using a Qiagen tissue extracting kit (Qiagen, USA) and following the manufacturer's directions, total RNA was extracted. Using a high-capacity cDNA reverse transcription kit (Fermentas, USA), the entire RNA was transformed into cDNA (0.5-2 g). The next step involved real-time qPCR multiplication and evaluation using an Advanced Biosystem and program version 3.1 (Step One TM, USA). The following were the reaction conditions: One cycle at 95°C for 30 seconds (20°C/sec), followed by 40 cycles at 95°C for 5 seconds (20°C/sec) and 60°C for 20 seconds (20°C/sec).

The proportion of the PCR products was estimated utilizing the 2 Ct techniques after standardization to beta actin. In Table 1, the primers are displayed.

2.7. Biochemical parameters in the serum

The concentrations of albumin and alanine aminotransferase in the blood serum were measured using an Au 400 automatic biochemical scanner (Olympus, Tokyo, Japan).

2.8. Analysis of the histopathology

The liver specimens were obtained, mended, and incubated in PBS containing 40 g of paraformaldehyde per liter for a whole night at 4 °C. Hematoxylin and Eosin (HE) were used to stain successive five-μm slices of the liver's right lobe in order to gauge the degree of fibrosis. A 200x magnification Olympus BX 53 light microscope and an Olympus DP73 CCD were used to capture the images, and then they were histo-pathologically evaluated.

2.9. Statistical methods

The statistical program SPSS version 22 was used to code and assess the data. The mean and standard deviation were used to summarize the data. When comparing more than two groups, an analysis of variance (ANOVA) was used in conjunction with a multiple comparisons post hoc test. In order to confirm correlations between quantitative variables, the Pearson correlation coefficient was used. A statistically significant difference was determined to exist at $P<0.05$ [24].

Table 1. Contains a real-time PCR primer sequence of TGF-β. As a housekeeping reference gene, β-actin was employed.

TGF-β	Forward-looking primer: 5-GTGGGAGAAAGTTTGCCAGG-3 Reverse-looking primer:5- GTAGGAAGAGAGGGAAGAGG-3
β-actin	Forward-looking primer:5-CCAGGCTGGATTGCAGTT-3 Reverse-looking primer: 5-GATCACGAGGTCAGGAGATG-3

3. RESULTS

3.1. Serum levels of biochemical parameters

It was found that IP CCl4 injection significantly increased the Alanine Amino Transferase (ALT) mean level (66.7±12.6) U/L in comparison with the control group (18±1.6) U/L (p-value < 0.001), as well as a significantly decreased mean level of albumin (3.4±0.49) gm/dl in comparison to the conventional placebo group (5.2±0.29) gm/dl (p-value < 0.001).

The IP administration of NaHS, whether alone or after IV administration of MSCs, significantly attenuated the mean level of ALT (36.5±4.6, 22.7±3.8) U/L compared with the CCl4 group (G2) and significantly increased the mean level of albumin (4±0.38, 4.7±0.32) gm/dl respectively, compared with G2.

The outcomes also demonstrate how well IV MSC therapy reduced the total mean level of ALT (30.2±2.6) U/L when compared with G2. A more pronounced decrease was obtained following the administration of BMSCs together with NaHS (22.7±3.8) U/L, and there is an increase in the mean level of albumin (4.1±0.49) gm/dl when compared with G2. A more pronounced increase was obtained following the administration of BMSCs with NaHS (4.7±0.32) gm/dl, as presented in Table 2.

Table 2. ALT (U/L) and Albumin (gm/dl) mean levels among Studied Groups.

	Normal (G1)	CCL4(G2)	H2S(G3)	MSCs(G4)	MSCs pretreated H2S(G5)
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
ALT(U/L)	18±1.6	66.7±12.6*	36.5+4.6*#	30.2±2.6#	22.7+3.8#
Albumin gm/dl	5.2±0.29	3.4±0.49*	4+0.38*	4.1±0.49*	4.7+0.32#

The relative expression of the transforming growth factor beta (TGF-β) gene was noticeably greater in the group with liver fibrosis caused by CCl4 (130±11.15) pg/mg than in the normal control group (48±8.2) pg/mg (p value < 0.001).

The IP administration of NaHS significantly attenuated the relative expression of the TGF-β gene (80±10.4) pg/mg, compared with G2.

The outcomes also demonstrate how well IV MSC therapy decreases the relative expression of the TGF-β gene (56.5±3.3) pg/mg when compared with G2. A more pronounced decrease was obtained following the administration of MSCs together with NaHS (48±4.1) pg/mg, as presented in Table 3.

Table 3. TGF gene expression by RT-PCR technique among studied groups.

	Normal(G1)	CCL4(G2)	H2S(G3)	MSCs(G4)	MSCs pretreated H2S(G5)
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
TGF pg/mg	48±8.2	130±11.15*	80+10.4*#	56.5±3.3#$	48+4.1#$

3.2. Histological Results (Hematoxylin and Eosin-Stained Sections)

- Control group: The control group displayed typical hepatic architecture in the form of the portal tract in the core, which was surrounded by a straightforward cubical epithelium-lined bile duct and portal vein. Hepatocytes are arranged in cords with pale vesicular nuclei and acidophilic cytoplasm (Fig. 1A).

- CCl4-induced liver fibrosis group: The bile duct and portal vein were enlarged and congested, according to sections from the fibrosis group. The vein between the hepatocytes had a significant

mononuclear infiltration. Few hepatocytes had dark nuclei (Figs. 1B & 1C).

- H2S-treated group: Hepatic sections demonstrated some improvement in fibrosis. The portal tract has a congested portal vein (black arrows), and bile duct (red arrows). Only a few cellular infiltrations (yellow arrows) are observed around the portal vein and in-between hepatocytes (curved arrows) (Fig. 1D).

- MSCs-treated group: Hepatic sections demonstrated some improvement in fibrosis. Exhibiting congested portal vein (black arrows), bile duct (red arrows) and mononuclear cell infiltration (yellow arrows). Hepatocytes are arranged in cords with a pale vesicular nuclei (curved arrows) (Fig. 1E).

- MSCs-pretreated H_2S group: Reveal normal hepatic architecture apart from minimal infiltration (Fig. 1F).

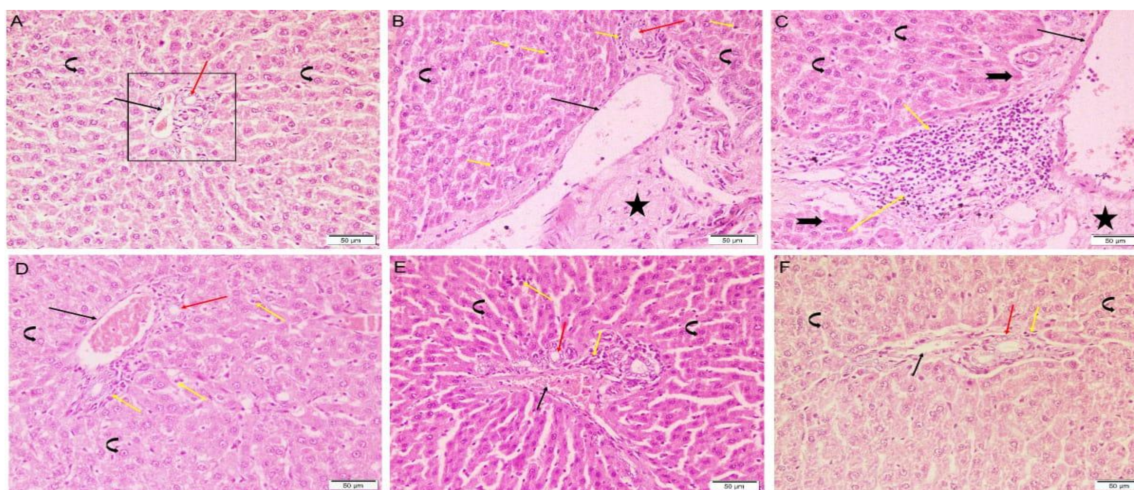

Figure 1. histopathological examination of rat's liver tissue (H&Ex400). (A)Showing normal hepatic structure (B&C) Showing large area of fibrosis in liver tissue (D&E) Showing some improvement of hepatic fibrosis (F) Showing normal hepatic architecture apart from minimal infiltration.

4. DISCUSSION

Liver fibrosis, which is primarily defined by excessive ECM buildup, is a harmful trait of numerous persistent liver conditions that are episodes of chronic liver damage [25]. Disorders of the liver are considered a major health problem [26]. Due to the lack of appropriate medications, nearly all chronic liver illnesses result in fibrosis, which is then followed by cirrhosis or hepatocellular carcinoma and causes over a million deaths annually [27].The transplantation of the liver is the only available therapy for liver cirrhosis at this time [9]. But, because of liver donor deficiency and immunological rejection and the complicated and high-cost surgery, researchers are developing a novel treatment strategy for people with liver fibrosis [7].

The present research looked into the potential anti-fibrotic effects of bone marrow-derived mesenchymal stem cells (BMSCs) and hydrogen sulphide (H2S) on liver fibrosis. Considering that sodium hydrogen sulphide (NaHS) preconditioning can improve stem cell expansion and lengthen the lifespan of stem cells and tissue cells used in therapy by increasing anti-oxidant defense, this study is a novel attempt to demonstrate the effectiveness of combining BMSCs and H2S in reducing rat liver fibrosis.

In order to completely examine the curative properties of MSCs and H2S on hepatic fibrosis and its primary mechanisms, we created a CCl4-induced hepatic fibrosis experiment using rats. The example, which is being extensively employed worldwide to investigate hepatic fibrosis mechanisms, is the most accurate representation of human hepatic fibrosis.

Stem cell treatment has emerged as an alluring choice to induce tissue renewal and hepatocyte repair [28]. MSCs can cure liver fibrosis in animal models [29]. MSCs, one of the numerous varieties of stem cells, have arisen as an intriguing study topic since they are simple to isolate, multiply well in vitro, have little immunogenicity, and pose no moral dilemmas [8]. They induce organ integrity because of their mitotic, anti-fibrotic, angiogenic, anti-apoptotic, and immunomodulatory qualities [30]. They can also travel to defective regions, develop into cells that resemble hepatocytes, and then swap out injured hepatocytes for healthy ones [28]. BMSCs are used currently to decrease hepatocytes' apoptosis and encourage their renewal because of

their high proliferative ability, differentiation potential, and lack of ethical problems [7].

In the present results, intravenous (IV) administration of BMSCs (G4) significantly attenuated the mean levels of ALT (30.2±2.6) U/L and significantly increased the mean level of albumin (4.1±0.49) gm/dl compared with the CCl4 group (G2) (66.7±12.6) U/L and (3.4±0.49) gm/dl, respectively ($P<0.05$).

The two most commonly used tests for identifying hepatic illness are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Despite their diagnostic limitations, they are commonly employed in patients to screen for possible liver disease, to check on patient safety while receiving medication, or in the context of occupational health surveys [31]. They are intracellular enzymes in cases of leakage into the blood circulation that indicate hepatocyte injury [32].

Albumin is a protein that indicates the synthetic function of the liver. In patients with liver fibrosis, protein synthesis, including albumin and various coagulation factors is decreased, and that leads to hypoproteinemia. Many events occur when the albumin level is decreased such as hepatic insufficiency, decreased immunity, increased infection occurrence, ascites, liver dysfunction, and so on [33].

Our results corroborated those of Abdel Aal et al. [34], who reported that rats fed CCl4 displayed significantly higher blood activities of ALT and AST ($P<0.001$). Additionally, there was a substantial drop in albumin amount in the serum ($P<0.05$). When contrasted with the CCl4 group, regularization of the blood activity levels of ALT and AST as well as serum albumin was seen in the MSCs and mesenchymal stem cells-conditioned media groups.

Also, the findings are supported by those of Sabry et al. [29], who discovered that bone marrow-derived mesenchymal stem cells-microvesicles (BMSC-MVs) have anti-inflammatory, pro-angiogenic, and anti-fibrotic capabilities that can help rats with liver fibrosis caused by CCl4 to resolve. When contrasted with the CCl4 fibrotic group, the group receiving BMSC-MVs treatment demonstrated a substantial drop in blood alanine transaminase (ALT) enzyme levels ($p<0.05$), however demonstrating a substantial rise in serum albumin levels ($p<0.05$).

Also, the findings corroborated those of Al-Dhamin et al. [7], who claimed that transplanting stem cells, including BMSCs, had been shown to be effective at treating fibrotic livers. By reducing blood levels of alanine and aspartate aminotransferases and increasing levels of albumin as compared to the untreated CCl4 group, BMSCs reduce hepatic fibrosis in vivo. Additionally, by restoring the composition and performance of the liver, BMSCs may improve the assessment of liver function and reduce the toxic impact of CCl4.

Abdelgwad et al. [35] reported that groups treated with BMSCs had higher albumin concentrations ($p <.05$) and lower ALT concentrations ($p <.05$) when compared to the CCl4 group that wasn't given any treatment.

In the present results, IV administration of BMSCs (G4) significantly reduced gene expression of TGF-β (56.5±3.3) pg/mg compared with the CCl4 group (G2) (130±11.15) pg/mg ($P<0.05$).

Although TGF expression is elevated in every phase of degenerative liver disease involving fibrosis, it continues to exist in healthy liver tissue. TGF-1 causes an excessive accumulation of ECM, activates HSC, and promotes liver fibrosis. Recent studies suggest that blocking the TGF-1 pathway may lower fibrogenesis [32]. It was demonstrated that ROS might operate as a TGF-β signalling inducer or effector and so create a cycle of fibrosis. Furthermore, increased TGF-β levels significantly accelerate the death of hepatocyte cells, resulting in chronic liver injury [36]. When kupffer cells are activated by CCl4 metabolism, their levels of intracellular calcium rise, and they release inflammatory cytokines that cause hepatocyte apoptosis. The most prevalent and potent oxidizing enzyme identified in monocyte granules and neutrophils is myeloperoxidase (MPO). It regulates profibrotic TGF-1 cytokine activity and promotes hepatocyte injury by boosting matrix metalloproteinases and triggering the production of hypochlorous acid (HOCl) [4]. It was also shown that activated HSCs are capable of producing TGF-1, which in turn induces the transcription of alpha smooth muscle actin (α-SMA) and other ECM proteins [14].

Our findings confirmed with those of Al-Dhamin et al. [7], who found that transplanting BMSCs decreased serum and hepatic levels of TGF-β1, decreased serum expression of Smad3, and raised Smad7 expression.

Also, the results are supported by those of Sa'diyah et al. [37], who claimed that MSCs have an immune-modulating capacity that may be used as a novel therapy for LF repair and regeneration through suppression of TGF-β, which enhances hepatocyte survival and prevents HSC activation. These claims suggest that MSCs may reduce TGF-β production by inflamed cells to promote the best liver regeneration in LF. On the seventh day

following MSC treatment, the level of TGF drops in LF rat model systems. When compared to the control group, the levels of TGF-β substantially dropped in the MSC groups ($p < 0.05$).

According to Altaib et al. [38], rats treated with BMSC-MVs had significantly lower serum TGF-β levels than the CCl4 fibrotic group. Further studies reported that BMSCs downregulated the expression of TGF compared to CCl4-induced fibrosis rats.

Also in our results, IP administration of an exogenous H2S donor (NaHS) (G3) significantly decreased mean levels of ALT (36.5+4.6) U/L and increased mean level of albumin (4+0.38) gm/dl, and reduced gene expression of TGF-β (80+10.4) pg/mg, compared with CCL4 group (G2) (66.7+12.6) U/L, (3.4+0.49) gm/dl, (130+11.15) pg/mg respectively ($p < 0.05$).

H2S plays a crucial regulatory role in various physiological and pathological issues. The pathophysiology of fibrosis, which is connected to a lack of H2S, causes structural and functional damage to a number of organs. In cirrhotic livers, decreased H2S generation results in enhanced resistance. H2S exerts a variety of effects, but its main inhibitory effects are on inflammatory indicators, oxidative stress, and lipid peroxidation, notably its stimulation of ATP-dependent potassium (KATP) channels and elevation of phosphorylated Akt expression. Additionally, H2S exhibits cytoprotective and vasodilatory properties. As a result, it might play a part in treating hepatic fibrosis [14]. Exogenously given H2S has been shown to promote wound healing [39].

According to our results, Wu et al. [40] showed that H2S donor (NaHS or Na2S) therapy at relatively low concentrations could reduce the quantity of ROS, peroxidation of lipids, and cytochrome P 450 2E1 (CYP2E1) activity while increasing the amount of glutathione (GSH) and anti-oxidative activity of enzymes. According to research studies, a CBS deficit increases oxidative stress, steatosis, and fibrosis in rodents' livers, showing that H2S plays a part in liver fibrosis. Additionally, recent research has demonstrated that H2S can reduce both in vivo and in vitro liver fibrosis. Due to this, the treatment of a number of fibrotic disorders may benefit from targeting H2S as a viable therapeutic target. NaHS administration at dose of 56 mol/kg/day prevents CCl4-induced liver fibrosis by lowering TGF-β1 expression and liver extracellular matrix sediment. Also, our findings are in concurrence with those of DiNicolantonio et al. [41] who claimed that exogenous hydrogen sulphide (H2S) reduces fibrosis in a variety of tissues in vivo and opposes TGF signaling in vitro. The fact that agents that inhibit the expression of these enzymes as well as genetic deficiencies in H2S-producing enzymes, like CBS and CSE, promote tissue fibrosis suggests that this effect is physiologically significant. Additionally, in fibrosis designs, the production of these enzymes is usually decreased. Similar findings were made by Liu et al.[42], who discovered that H2S protects against liver cirrhosis, CCl4-induced hepatotoxicity, nonalcoholic steatohepatitis, and hepatic ischemia/reperfusion injury. Exogenous H2S reduced the degenerative changes to the liver and the elevated AST and ALT blood levels in rats that had consumed Paraquate. Also, our findings corroborated those of Wang et al.[25], who discovered that NaHS therapy has been shown to inhibit the stimulation of the TGF-β/Smad cascade to start anti-inflammatory characteristics. H2S also lessens the phosphorylation of p38 MAPK, which inhibits the circuit and increases anti-apoptotic and anti-oxidative characteristics. Pro-apoptotic and ROS activities are suppressed as a result of H2S's additional inhibition of the Wnt/catenin cascade's induction. Through the prevention of TGF-β-mediated collagen accumulation, H2S can lessen network buildup and cardiac fibrosis in rats with diabetes. This in turn causes a reduction in type 1 and type 3 collagen along with heart fibrosis.

As a way to increase the MSC therapy's capacity for healing, a novel suggestion is offered. Combining methods may be exciting and aid in understanding MSCs' potential, functions, and therapeutic perspectives [43, 44]. In cases of CCL4-induced damage to the liver, minor adjustments to stem cell therapy have improved the liver's function. Stem cell therapy must be modified in order to improve its efficacy and rate of success [15, 45].

Additionally, our findings demonstrate the value of preconditioning MSCs with NaHS. A more pronounced decline in the mean levels of ALT (22.7+3.8) U/L and an increase in the mean level of albumin (4.7+0.32) gm/dl after administration of MSCs pretreated with H2S than administration of each of them alone in comparison with the CCl4 group (G2) (66.7+12.6) U/L and (3.4+0.49) gm/dl, respectively. Also, there is a more pronounced decline in the gene expression of TGF-β (48+4.1) pg/mg compared with the CCL4 group (G2) (66.7+12.6) pg/mg ($p < 0.05$).

According to Abdelmonem et al.'s [46] research, which supports our findings, transforming growth factor plays a critical role in the pathophysiology of cardiac fibrosis and remodeling. When compared to the group of patients with heart failure (HF), NaHS therapy resulted in a considerable drop in TGF-1 levels and fewer degenerative changes in damaged

heart tissue. The group treated with BMSC displayed a slight rise in TGF-1 levels and slight fibrosis in comparison to the HF group. The combined use of BMSCs with NaHS led to a more pronounced diminution in fibrosis than the HF and BMSC-treated groups, as shown by a significant drop in TGF-1 levels and a nearly typical histological organization of the cardiac bundle that is histologically intact and has nuclei that are positioned in the middle (close to normal). Similar to the group of HF, the group of NaHS-preconditioned BMSCs showed degenerative alterations, including a substantial drop in TGF-1 levels and moderate fibrosis with peripherally situated nuclei.

Additionally, the outcomes supported the findings of Buonvino et al.[13], who observed that H2S has been shown to increase cell survival by triggering a number of molecular signalling pathways and may be crucial in controlling the homeostasis and stemness of MSC. Significantly, myocardial infarction repair in rats after engraftment with MSC preconditioned with H2S. In addition, in hypoxic-ischemic settings, preconditioning cardiac MSC and BMSC with external H2S increased their ability to heal by encouraging growth and decreasing cytotoxicity. As a result, H2S preconditioning of stem cells may improve their viability and effectiveness for reconstructing and regenerating tissues when embedded in scaffolds.

In summary, the current study's findings suggest that BMSC and H2S therapy may reduce the expression of the TGF-β gene, and they could potentially reduce the rises in serum ALT levels caused by CCl4 and the CCl4-induced decreases in serum albumin. Additionally, they increased hepatocyte regeneration and decreased hepatocyte death. These findings suggested that the rat liver fibrosis model caused by CCl4 could be effectively treated by pretreating BMSC with NaHS . Collectively, the synergistic effects of NaHS and BMSCs in reducing liver damage may be attributed to the stimulating impact of hydrogen sulfide on the homing of BMSCs to the liver, their development, and their transformation into hepatocytes with neovascularization.

5. CONCLUSION

The current results clearly indicate the beneficial effects of MSCs preconditioned with H2S in protection against liver fibrosis. Depending on the findings of the present study, it could be suggested that MSCs pretreated with H2S may exert their anti-fibrotic action through their inhibition of TGF-β, and that they can also have antioxidant and anti-inflammatory potentials by preventing the deposition of extracellular matrix.

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Conflicts of Interest: The authors have declared no conflicts of interest.

Ethical Statement: The Al-Azhar University's ethics for committee for the pharmacy faculty's female students gave the study their blessing (code: 156; session 18 on 18/12/2017).

Author Contribution: Sawsan A. Abd El Mohsen: PCR amplification, data collection and wrote the manuscript, Laila A. Rashed: supervised plane of the work and reviewed the manuscript, Seham M. Saied El Nakeeb: revised the molecular bases of the work and reviewed the manuscript, Doha El-Sayed Ellakwa: contributed to the data collection, editing and writing of the publication.

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