RESEARCH PAPER

Human Mesenchymal Stem Cell Secretome Increases Catalase In Wistar Rats With Cholestasis

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ABSTRACT

Background: Cirrhosis represents the end stage of chronic liver disease and remains a major cause of morbidity and mortality worldwide. While liver transplantation is the only curative option, limited donor availability and high costs restrict accessibility. Ursodeoxycholic acid (UDCA) is often used to delay disease progression, but its regenerative capacity is limited. Mesenchymal stem cells (MSCs) and their secretome have been proposed as novel therapies, primarily through their paracrine antioxidant, anti-inflammatory, and anti-apoptotic effects. Oxidative stress plays a central role in cholestasis, and catalase serves as a critical enzyme in counteracting hydrogen peroxide–induced injury. This study aimed to investigate the effect of human MSC-secretome (HuMSC-S) on catalase levels in a rat model of cholestasis.

Methods: Twenty-four male Wistar rats (200–250 g) underwent common bile duct ligation to induce cholestasis and were randomly assigned into four groups: K1 (control), K2 (UDCA 4.5 mg/week), K3 (HuMSC-S 0.2 ml/kg/week), and K4 (UDCA + HuMSC-S). Interventions were administered for 4 weeks. Catalase levels were measured using ELISA. Statistical analysis included Shapiro–Wilk for normality, one-way ANOVA, and post hoc LSD test.

Results: Catalase activity significantly differed among groups ($p < 0.001$). The control group (1.46 ± 0.06 U/mL) showed the lowest levels. UDCA increased catalase to 4.89 ± 0.16 U/mL, while HuMSC-S further enhanced it to 6.79 ± 0.41 U/mL. The combination group demonstrated the highest activity (7.99 ± 0.29 U/mL), significantly exceeding all other groups.

Conclusion: HuMSC-S administration significantly enhanced catalase levels in cholestatic rats, with the greatest effect achieved in combination with UDCA. These findings suggest a synergistic antioxidant and hepatoprotective effect, supporting the therapeutic potential of MSC-secretome as a promising adjunct to conventional therapy.

Keywords: Catalase; Mesenchymal stem cell secretome; Ursodeoxycholic acid; Cholestasis

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BACKGROUND

Chronic liver disease that progresses to cirrhosis remains a major global health problem, with high morbidity and mortality rates worldwide. The underlying causes include viral infections, autoimmune mechanisms, biliary obstruction, and various metabolic abnormalities.[1] Cirrhosis is often irreversible, and liver transplantation is currently the only curative therapy; however, donor availability and high costs limit its accessibility.[2] As a result, pharmacological options such as ursodeoxycholic acid (UDCA) are commonly used to delay disease progression, but their regenerative capacity is limited and may even interfere with cellular repair mechanisms in some conditions.[3]

Mesenchymal stem cells (MSCs) have attracted increasing attention as an alternative therapy due to their low immunogenicity, self-renewal ability, and capacity to secrete bioactive molecules that promote tissue repair.[4,5] Instead of direct cell replacement, the therapeutic potential of MSCs is now understood to rely largely on their secretome, a collection of soluble proteins, lipids, and extracellular vesicles that modulate inflammation, inhibit apoptosis, and enhance regeneration.[5] In liver injury models, the MSC secretome has been shown to reduce hepatocyte death, promote antioxidant defenses, and improve histological outcomes.[6]

Oxidative stress plays a central role in the progression of cholestasis and subsequent fibrogenesis. Excessive production of reactive oxygen species (ROS) contributes to lipid peroxidation, mitochondrial dysfunction, and activation of hepatic stellate cells, which promote fibrosis.[7,8] Antioxidant enzymes such as catalase are critical in neutralizing hydrogen peroxide, thereby protecting hepatocytes from ROS-induced injury. In

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experimental cholestasis, reduced catalase activity correlates with more severe oxidative damage and liver dysfunction. Thus, enhancing catalase expression or activity could represent a protective strategy against oxidative liver injury.

Given this background, the present study aims to investigate the effect of human MSC-secretome administration on catalase levels in Wistar rats with cholestasis. By focusing on catalase as a key antioxidant defense mechanism, this research may provide insights into the hepatoprotective role of MSC-derived secretome and its potential as an adjunct to conventional therapies.

METHODS

Study Design

Randomized experimental research with a post-test only control group design was carried out from April to December 2022 at Gadjah Mada University Experimental Animal Laboratory.

Intervention

The UDCA dose for this study was based on the range of doses used in human subjects that were converted to experimental animals, resulting in a dose of 4.5 mg in rats weighing 20 grams.[9]

Human mesenchymal stem cell secretome (Hu-MSC-S) was manufactured at the Laboratory of Gadjah Mada University, Yogyakarta. The secretome was processed through four phases of culture, derived from human umbilical cord tissue harvested at 60% confluence using the warm trypsinization method.[10] The dose of Hu-MSC-S used was 0.2 ml/kg, based on effective doses reported in previous regeneration studies.[11]

Animal Models and Study Procedures

Twenty-four male Wistar rats (aged 2 months, weighing 200–250 g) were used as experimental models. Rats were adapted for 5 days and maintained with food and water ad libitum. All procedures were conducted in accordance with animal welfare regulations. Blood samples from the rats' tails were collected prior to intervention to obtain baseline measurements. For surgical induction of cholestasis, anesthesia was given with ketamine hydrochloride (0.5 cc, IM), and cefotaxime (18 mg, IV) was administered as prophylaxis. After disinfection, a midline incision was made to expose the peritoneum and liver. The common bile duct was isolated from the portal vein and hepatic artery using micro-serrated forceps, then ligated with 4-0 silk sutures to achieve obstruction. The incision was closed with layered suturing. After 2 weeks, rats were randomly divided into four groups: K1: Control (no treatment); K2: UDCA (4.5 mg peroral, once weekly for 4 weeks); K3: Hu-MSC-S (0.2 ml/kg intraperitoneal, once weekly for 4 weeks); K4: Combination UDCA + Hu-MSC-S (same doses as above). At the end of treatment, all rats were sacrificed using cervical dislocation under general anesthesia.

Evaluation

Catalase levels were measured from rat serum obtained via venous blood samples. Assessment was performed using an ELISA-based method according to the manufacturer's protocol. The results were expressed in U/mL.

Data Analysis

Data were first tested for normality using the Shapiro–Wilk test. For normally distributed data, analysis was performed using One Way ANOVA, followed by Post Hoc Least Significant Difference (LSD) to assess pairwise differences between groups. A p value < 0.05 with 95% confidence interval was considered statistically significant.

Ethical Clearance

The experiments were conducted following institutional guidelines and were approved by the Health Research Ethical Committee, Faculty of Medicine, Universitas Diponegoro (Protocol Number: 09/EC/H/FK-UNDIP/1/2023). All animal handling complied with animal welfare regulations.

RESULTS

All of the rat's body weight was measured on the 6th day of acclimatization, which was also the first day of the treatment process. The analysis result showed a normal and homogeneous distribution of the body weight of the rats (Table 1).

Table 1. Baseline Body Weight

Group	Mean ± SD (gram)	Median (min – max)	p£	Levene Statistic
K1	191.57 ± 3,36	191 (188 – 197)	0.212*	0.735**
K2	191.71 ± 3,25	191 (188 – 197)	0.610*	
K3	186.57 ± 3,41	187 (182 – 191)	0.760*	
K4	189.86 ± 2,41	190 (186 – 193)	0.976*	

Description: *Normal ($p > 0.05$) £Shapiro-wilk, **Homogeny ($p > 0.05$)

Descriptive analysis of catalase levels in all experimental groups is presented in Table 2. The mean catalase level in the control group (K1) was 1.46 ± 0.06, while in the UDCA-treated group (K2) it increased to 4.89 ± 0.16. A further increase was observed in the HuMSC-S group (K3) with a mean of 6.79 ± 0.41, and the highest levels were found in the combination therapy group (K4), reaching 7.99 ± 0.29. The Shapiro–Wilk test confirmed that the distribution of catalase levels in all groups followed a normal pattern ($p > 0.05$). Similarly, Levene's test showed homogeneity of variances ($p = 0.081$), confirming that the data met the assumptions for parametric analysis.

Table 2. Normality and homogeneity of catalase levels

Group	Mean ± SD	Median (min – max)	p	Lavene
K1	1,46 ± 0,06	1,47 (1,38 – 1,53)	0,897*	0,081**
K2	4,89 ± 0,16	4,92 (4,61 – 5,03)	0,311*	
K3	6,79 ± 0,41	6,85 (6,24 – 7,22)	0,248*	
K4	7,99 ± 0,29	8,06 (7,47 – 8,25)	0,170*	

Description: * Normal (p > 0,05) ; ** Homogen (Levene > 0,05)

The results of one-way ANOVA revealed a statistically significant difference in catalase levels among the four groups ($p < 0.001$) (Table 3). This indicates that the administration of UDCA, HuMSC-S, or their combination significantly influenced catalase activity in Wistar rats with cholestasis.

Table 3. ANOVA results of catalase levels

Group	Mean ± SD	p
K1	1,46 ± 0,06	<0,001*
K2	4,89 ± 0,16	
K3	6,79 ± 0,41	
K4	7,99 ± 0,29	

Description: * Significant (p < 0,05)

Post hoc LSD tests provided more detailed insights into intergroup comparisons (Table 4). Catalase levels in the UDCA group (K2) were significantly higher compared to the control group (K1) (mean difference = 3.43; $p < 0.001$). An even greater increase was observed in the HuMSC-S group (K3), which demonstrated significantly higher catalase levels compared to both the control group (K1; mean difference = 5.33; $p < 0.001$) and the UDCA group (K2; mean difference = 1.90; $p < 0.001$). Importantly, the combination group (K4) showed the most pronounced effect, with catalase levels significantly higher than all other groups, including HuMSC-S alone (mean difference = 1.20; $p < 0.001$). These findings suggest a synergistic effect between UDCA and HuMSC-S in enhancing catalase activity.

Table 4. Post hoc LSD test of catalase levels

Group		Mean Difference	p
I	II		
K1	K2	-3.425	<0,001*
	K3	-5.328	<0,001*
	K4	-6.528	<0,001*
K2	K1	3.425	<0,001*
	K3	-1.903	<0,001*
	K4	-3.103	<0,001*
K3	K1	5.328	<0,001*
	K2	1.903	<0,001*
	K4	-1.200	<0,001*
K4	K1	6.528	<0,001*
	K2	3.103	<0,001*
	K3	1.200	<0,001*

Description: *Significant (p<0,05)

The boxplot (Figure 1) illustrates the distribution of catalase levels across groups. A clear stepwise increase is observed, starting from the control group with the lowest levels, followed by the UDCA group, the HuMSC-S group, and finally the combination group with the highest levels. The narrow range of values within each group further highlights the consistency of the results, with minimal overlap between groups, supporting the statistical findings from ANOVA and post hoc analyses.

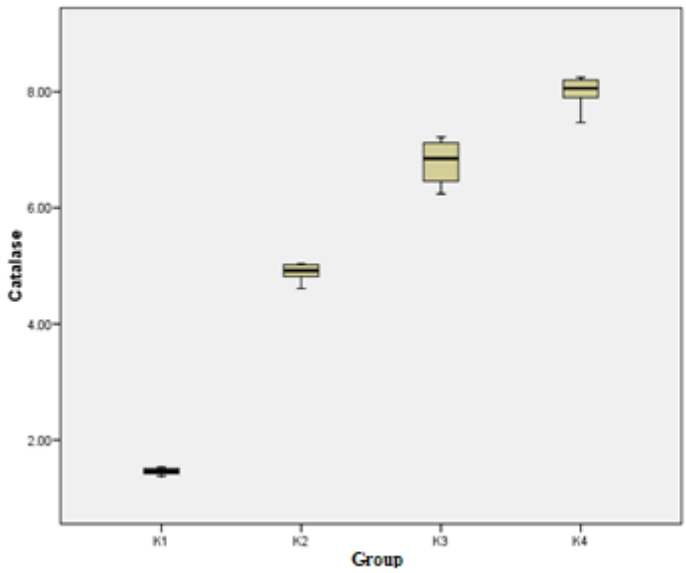

Figure 1. The boxplot graphic of catalase levels in each group

Overall, these results demonstrate that cholestasis significantly decreases catalase activity, while therapeutic interventions with UDCA, HuMSC-S, and particularly their combination are able to restore and even enhance catalase levels. The most effective treatment in improving antioxidant defense, as reflected by catalase activity, was the combined administration of UDCA and HuMSC-S.

DISCUSSION

This study demonstrated that administration of human mesenchymal stem cell secretome (HuMSC-S) significantly increased catalase levels in Wistar rats with cholestasis. The combination of HuMSC-S and UDCA produced the highest catalase activity, suggesting a synergistic effect in enhancing antioxidant defense mechanisms. Catalase is a critical antioxidant enzyme that neutralizes hydrogen peroxide and protects hepatocytes from oxidative stress. In the context of cholestasis, where bile duct obstruction amplifies reactive oxygen species (ROS) production, upregulation of catalase plays a pivotal role in preventing oxidative damage and fibrogenesis.

The observed hepatoprotective effect of HuMSC-S aligns with earlier studies highlighting the regenerative and antioxidant potential of MSC-derived secretome. Ignat et al. (2021) emphasized that MSC-secretome contains growth factors, cytokines, and extracellular vesicles that exert anti-fibrotic, anti-inflammatory, and antioxidant actions, making it a promising therapy for liver fibrosis.[12] Similarly, Basir et al. (2022) reported that human umbilical cord MSC-conditioned medium alleviated oxidative and inflammatory stress in a CCl_4-induced acute liver injury model, further supporting its protective role against ROS-mediated damage.[13] Our findings extend these observations by showing that MSC-secretome not only reduces oxidative stress but also specifically enhances catalase activity in a cholestatic setting.

Another possible mechanism is related to the paracrine signaling of MSC-secretome, which contains extracellular vesicles capable of transferring antioxidant enzymes and regulatory RNAs. Varderidou-Minasian & Lorenowicz (2020) described that MSC-derived extracellular vesicles are instrumental in modulating oxidative stress responses and tissue repair.[14] Moreover, Ferreira et al. (2018) highlighted that pre-conditioning MSCs could further optimize their secretome profile, enriching antioxidant and cytoprotective factors.[15] These insights suggest that the elevation of catalase levels in our study may be mediated not only by stimulation of endogenous antioxidant pathways but also by the direct transfer of antioxidant molecules via extracellular vesicles.

The synergistic effect observed in the combination group (UDCA + HuMSC-S) deserves particular attention. While UDCA is widely used as a standard therapy in cholestatic liver disease for its cytoprotective and anti-apoptotic effects[16], its regenerative potential has been questioned. Kotb (2012) reported that UDCA may interfere with cell proliferation and regeneration by altering DNA repair mechanisms.[3] However, when combined with HuMSC-S, our results indicate an additive or even synergistic effect, reflected in the highest catalase levels. This suggests that MSC-secretome may compensate for the limited regenerative capacity of UDCA, while UDCA may contribute to stabilizing the cellular environment and facilitating the action of secretome-derived factors.

Our findings are also consistent with the broader literature on MSC therapy in liver disease. Gao et al. (2022) emphasized that MSC-based interventions can slow disease progression and enhance antioxidant responses in chronic liver injury.[17] Yuan et al. (2022) further showed that MSC homing improves therapeutic efficacy in liver models, largely through paracrine signaling.[18] In addition, González-González et al. (2020) and Han et al. (2022)

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described the MSC secretome as the cornerstone of cell-free regenerative medicine, particularly because of its anti-apoptotic, antioxidant, and immunomodulatory properties.[19,20] Together, these findings support our interpretation that secretome therapy is highly effective in restoring antioxidant balance, with catalase serving as a key indicator of hepatocyte protection.

This study has several limitations. First, the sample size was relatively small, which may restrict the generalizability of the findings. Second, the assessment was limited to catalase levels as a marker of oxidative stress, without evaluating other antioxidant enzymes such as superoxide dismutase or glutathione peroxidase. Third, the molecular mechanisms underlying the observed increase in catalase were not directly investigated, leaving uncertainty about whether the effect was due to endogenous upregulation or direct transfer of antioxidant factors from the secretome.

Future research should expand the sample size and include additional biomarkers of oxidative stress and inflammation to provide a more comprehensive understanding of liver protection. Molecular analyses such as gene expression profiling or proteomics would help clarify the pathways involved in catalase upregulation. Moreover, preclinical studies using different models of liver injury, followed by well-designed clinical trials, are necessary to validate the translational potential of MSC-secretome, both as a monotherapy and in combination with UDCA, for cholestatic liver disease.

CONCLUSION

Human mesenchymal stem cell secretome (HuMSC-S) significantly enhanced catalase levels in Wistar rats with cholestasis, indicating a strong antioxidant and hepatoprotective effect. The combination of HuMSC-S and UDCA produced the greatest improvement, suggesting a synergistic interaction in restoring redox balance. These findings highlight the potential of MSC-secretome as a promising cell-free therapeutic strategy for cholestatic liver disease, either as a stand-alone treatment or in combination with existing standard therapies.

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