EFFECT OF UMBILICAL CORD MESENCHYMAL STEM CELLS WITH/WITHOUT SILYMARIN ON APOPTOSIS, IMMUNOMODULATION, PROLIFERATION, AND NECROSIS OF HEPG2 CELLS

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ABSTRACT

Introduction: Liver cirrhosis and liver cancer are the main problems of liver disease, and liver cirrhosis is the 11th leading cause of death worldwide. Fibrosis is a progressive process of liver disease before cirrhosis. Currently, stem cells are designed to treat various conditions, including chronic liver disease. Stem cells can inhibit the progression of fibrosis based on their immunomodulation, anti-apoptotic and proliferative effects.
Methods: This study was an in vitro experimental study. Mesenchymal stem cells (MSC) were taken from the umbilical cord of newborns. This study used the HepG2 cell line because it has the characteristics of hepatocyte cells. Cell lines were divided into four groups. The examination was performed on Nuclear factor kappa B (NF-κB), caspase-3, apoptosis, necrosis, and proliferation.
Results: Mesenchymal stem cells have the effect of reducing NF-κB levels, increasing caspase-3 levels, reducing necrosis events, and increasing proliferation in HepG2 cells. Silymarin decreases NF-kB levels, caspase-3 levels, apoptosis rate, and HepG2 cell proliferation but increases necrosis rate. Combining mesenchymal stem cells with silymarin can increase caspase-3 levels and reduce the incidence of necrosis in HepG2 cells.
Conclusion: Mesenchymal stem cells can regulate immunomodulation, increase proliferation, reduce necrosis, and regulate apoptosis of HepG2 cells. Combining mesenchymal stem cells with silymarin did not provide better results than treatment with mesenchymal stem cells as monotherapy. Further research is needed to assess mesenchymal stem cells' effectiveness in managing liver fibrosis.

Keywords: Mesenchymal stem cells, HepG2 cell line, Silymarin, Liver Fibrosis

INTRODUCTION

Liver disease causes 3.5% of deaths worldwide each year. Many etiologies cause liver disease, including viral hepatitis, alcoholism, steatohepatitis, and autoimmune processes. *Fibrosis* is the primary and most common process in chronic liver disease before cirrhosis [1,2]. During the process of liver fibrosis, there is an accumulation of extracellular matrix, especially type I and type III collagen, which will cause scarring and liver fibrosis [3]. Several treatments for cirrhosis have been carried out using different methods, ranging from surgery (transplantation/resection), several herbs to prevent or improve fibrosis, and addressing aetiology, but maximum results have not been obtained. There are still many complications, and the death rate is also increasing [4,5].

Cell-based therapy with mesenchymal stem cells (MSC) has several clinical benefits in treating patients with hepatic fibrosis. MSC is a multipotent cell that can be isolated from various tissues in vitro. The therapeutic benefits of MSC include self-renewal, engraftment, immunomodulation, multilineage differentiation, and regeneration [2,6,7]. MSCs are multipotent stem cells that originated from the mesoderm. MSC was first discovered in the bone marrow and then isolated from various human tissues such as adipose tissue, nerve tissue, umbilical cord, and amniotic fluid [7]. This study used stem cells from the umbilical cord to form MSC. Umbilical cord–mesenchymal stem cells (UC-MSC) are mainly located in the subcortical endothelial cord and perivascular area. Wharton's Jelly (WJ) mainly consists of porous structures lined with collagen fibres, proteoglycans, and embedded stromal cells [8]. Flow cytometry Analysis of WJ showed that UC-MSC expressed high CD24 and CD108. At the same time, the expression of specific markers FAP and CD40 was deficient, which indicates that WJ is a region rich in MSC [9]. UC-MSC can express characteristic markers of various cells. Besides expressing MSC markers (CD 105, CD 90, CD73), UC-MSC also expresses adhesion molecule markers (CD54, CD13, CD29, CD44) [10,11]. Initially, MSC was characterized by a population of cells capable of differentiating into osteoblasts, adipocytes, and chondrocytes in vitro. Further studies have reported that MSC differentiates into the skin, retinal pigment epithelium, lung, hepatocytes, renal tubular cells, and pancreatic cells [10].

Silymarin is an extract from milk thistle seeds used for decades to treat liver fibrosis. Preclinical studies have shown that silymarin has antioxidant and hepatoprotective properties [12]. The four main flavonolignan isomers in silymarin are silybin A, silybin B, isosilybin A, and isosilybin B. Silymarin protects liver cells through various mechanisms. First, stabilization of its plasma membrane through inhibition of fat peroxidation so that it helps fat retain its protective antioxidant in the form of glutathione [13,14]. Silymarin can also protect the liver from damage by inhibiting Tumor necrosis factor alpha (TNF-α), Interferon-gamma (IFN-γ), Interleukin 2 (IL-2), and Interleukin 4 (IL-4) as a consequence of inhibiting NF-κB activation. In addition, Silybin also has anti-inflammatory and anti-fibrosis effects. This study examined the effects of immunomodulation, proliferation, necrosis and apoptosis of mesenchymal stem cells with or without silymarin on HepG2 cells [14–17]

METHODS

This research was an in vitro study with an experimental research design. MSCs are produced from the umbilical cord of newborns (48 hours), about 12 cm long, which are then produced/isolated in Prodia's Stem Cells laboratory (ProStem). ATCC, USA reproduced hepG2 cells (ATCC, HB-8065). The methods and protocols used in this study are available as a collection in protocol.io DOI: dx.doi.org/10.17504/protocols.io.3byl4qke8vo5/v1.

Ethics considerations

After assessing the proposed research design, the Health Research Ethics Committee of Dr. Moewardi Hospital declared this study ethically approved on August 16, 2021, with reference number 178/I/HREC/2020.

UC-MSC culture

Umbilical cord samples were taken from the umbilical cord's healthy donor and were processed within 48 hours after cesarean section. Umbilical cords were delivered in a sterilized bottle. Under the biosafety cabinet, cords were washed with phosphate buffer saline (PBS) (Lonza17-516F) to remove any containing blood. Vessels

were removed, cut the cord into small pieces, and placed in a culture dish in a growth medium. UC-MSC were cultured in a 100 mm petri dish (NUNC,150464-CS) using growth medium DMEM (Lonza,12-917F) + 5% HPL (Sigma-Aldrich, SCM152) for five days until a confluence of > 80% was reached. The addition of TryPLE (Gibco,12605028) removed tissues. The solution was centrifuged (Thermo Scientific, 41330587) for 5 minutes at 500 g speed to obtain a cell suspension. A 1 ml PBS (Lonza17-516F) solution was added for cell count.

Immunophenotyping

The immunophenotyping process begins with the addition of 100 µL of cell samples to each well, then divided into three groups,

- Group 1, Nothing added to an unstained tube
- Group 2, Add 20 µL isotype positive and 20 µL isotype negative to the isotype tube
- Group 3, Add 20 µL cocktail positive and 20 µL cocktail negative to the cocktail tube

Samples were incubated in the dark for 30 minutes. Then, 1 mL PBS solution was added and centrifuged at 500 g for 5 minutes; the supernatant was discarded and analyzed using a flow cytometer.

HepG2 cell culture

HepG2 cells were cultured in a 100 mm petri dish (NUNC,150464-CS) using DMEM (Lonza,12-917F) + 5% HPL (Sigma-Aldrich, SCM152) medium for five days until it reached a confluence of >80%. When it reached confluency, the cells were harvested by adding 3 ml of TrypLe (Gibco,12605028) solution, then incubated at 37 ℃, 5% CO2, for 7 minutes. The sample was centrifuged for 5 minutes at 500 g speed to obtain a cell suspension. The supernatant was discarded, and 1 ml PBS (Lonza17-516F) solution was added for the cell count.

Proliferation, necrosis, apoptosis and immunomodulation test of HepG2 cells

A 100 µl HepG2 cell suspension solution was grown in 96-well plates (NUNC,163320) at 5x103 cells/wells density. Incubation occurred at 37ºC; 5% CO2 for 24 hours. Then, 100 ml of 1 mM oleic acid (Sigma-Aldrich, O1008) was added to each well to trigger cell fibrosis. Cells were incubated for 24 hours at 37ºC; 5% CO2. The study subjects were divided into four groups:

(a) The control group, which was not given any treatment (Cb)
(b) Silymarin group, which was given silymarin (Sigma-Aldrich, SCM152) with 250 mcg/ml solution (C1)
(c) UC-MSC group with a 1:1 ratio in DMEM (Lonza,12-917F) growth medium (C2)
(d) UC-MSC group in silymarin carrier solution (C3).

After adding the treatment, the cells were incubated at 37ºC; 5% CO2 for 24 and 48 hours. The test and control solutions were discarded, and 10 µl of MTT reagent with a 5 mg/mL concentration was added into each well. The cells were incubated again at 37 oC, 5% CO2, for 4 hours. MTT solubilization solution reagent (Sigma-Aldrich, M5655-1G) was added as much as 100 µl into each well. The absorbance was measured at a wavelength of 450 nm using an ELISA (Bio-Rad, 1681000XTU) reader for proliferation measurement.

Apoptosis and necrosis assay was started by adding 100 µl of RealTime-GloTM Annexin V Apoptosis and Necrosis Assay solution to each well; luminescence and fluorescence were measured at 500-550 nm intensity using a GloMax microplate reader. NF-kB and Caspase-3 examination was started by adding 100 µl of 1x cell extraction buffer PTR. Cells were detached using a mini cell scrapper. Cells were collected in a 1.5 ml microcentrifuge tube. A total of 50 microliters of cells were then inserted into each well on 96 well plates (NUNC,163320); at the same time, Lyophilized NF-kB p65 control lysate (pS536 + Total) and cleaved caspase-3 (Asp175) lyophilized standard (ab220655) were prepared by gradual dilution. 50 µl of the standard was added to the first two columns of 96-well plates. Add 50 µl of antibody cocktail for NF-kB (Abcam, ab133112) and caspase to each sample and standard. Incubation was carried out at room temperature for 1 hour. The absorbance was reading at 450 nm wavelength.

Data analysis

The data were presented as mean ± SD, then analyzed by SPSS 27 for Windows with a p-value <0.05, considered statistically significant.

RESULTS

UC-MSC culture and immunophenotyping

This study showed markers CD 73+, CD 90+, CD 105+, and Lin – after immunophenotyping. The expression of CD 73+ and CD 90+ was 99.9%, while CD 105+ was 98.5% (Table 1). UC-MSC conditioned media (CM) characteristics were obtained with Stromal cell-derived factor 1 (SDF-1), Brain-derived neurotrophic factor (BDNF), and IL-6 as dominant parameters. In the characteristics of the UC-MSC CM, the most significant expression was IL-6 of 1166.4 pg/ml, followed by SDF-1 expression of 861.95 pg/ml and BDNF of 126.60 pg/ml (Table 2). Stem cell cultures can also develop into fibroblast cells (mesoderm) in this study.

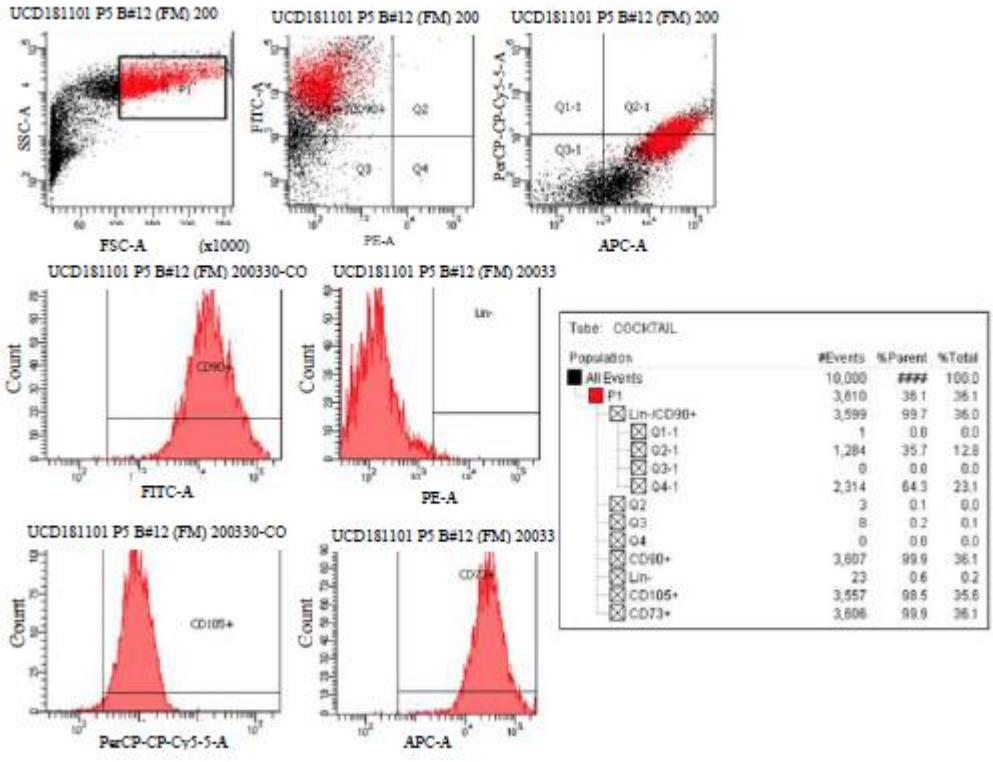

Figure 1. MSC Characterization

Table 1. Immunophenotyping of Mesenchymal Stem cells

Marker	Expression
CD73+	99.9%
CD90+	99.9%
CD105+	98.5%
Lin -	0.6%

Table 2. UC-MSC CM Identification

Variable	Result (pg/ml)
BDNF	126.60
SDF-1	861.95
FGF	48.13
VEGF	12.02
PDGF	1.33
EGF	6.25
NGF	31.52
IL-10	15.40
IL-1β	2.64
IFN-γ	4.06
MCP-1	0.08
IL-6	1166.4
IL-12p70	30.06
IL-17A	4.65
IL-18	13.44
IL-23	22.06

Immunomodulation effect

One of the immunomodulation processes is mediated by NF-κB. Therefore, in this study, NF-κB was used as one of the parameters to assess the immunomodulating effect after the administration of MSC with or without silymarin. The research data showed that the lowest NF-κB levels were in the C2 with an average NF-κB level of 0.103 ± 0.008 pg/ml, while the group with the highest NF-κB levels was in the Cb with an average level of NF-κB was 0.188 ± 0.012 pg/ml (Table 3). At 48 hours after treatment, C1 and C3 experienced a decrease in NF-κB levels, while C2 and Cb experienced an increase in NF-κB levels. The difference in NF-κB levels at 24 and 48 hours of treatment was different and statistically significant with a p-value <0.05. After 48 hours of treatment, the lowest NF-κB level was found in C1 0.096 ± 0.006 pg/mL, while the highest was in the Cb 0.218 ± 0.015 pg/mL. Comparative tests of each group showed that the three treatment groups significantly reduced NF-κB levels at 24 and 48 hours of treatment compared to the control group with a p-value <0.05 (Table 4).

Table 3. Simultaneous Difference Test for NF-κB Variables Based on Treatment Preparations

Groups	NF-κB	
	24 hours of treatment (Mean ± SD)	48 hours of treatment (Mean ± SD)
C1	0.108 ± 0.013	0.096 ± 0.006
C2	0.103 ± 0.008	0.112 ± 0.011
C3	0.107 ± 0.007	0.098 ± 0.007

Cb	0.188 ± 0.012	0.218 ± 0.015
p-value	< 0.001	< 0.001

Table 4. Partial Difference Test (Post Hoc) Variable NF-κB

Groups	NF-κB	
	24 hours of treatment (p-value)	48 Hour of treatment (p-value)
Cb vs C1	<0.001	<0.001
Cb vs C2	<0.001	<0.001
Cb vs C3	<0.001	<0.001
C1 vs C2	0.455	0.009
C1 vs C3	0.960	0.687
C2 vs C3	0.486	0.024

Apoptotic effect

Caspase-3 is a lysosomal enzyme involved in the apoptotic pathway and a protein that detects cell apoptosis. After 24 hours of treatment, the highest levels were found in the C3, 0.075 ± 0.007 pg/ml, and the lowest in the Cb, 0.071 ± 0.007 pg/ml. After 48 hours of treatment, the highest caspase-3 levels were found in the C2, 0.068 ± 0.004 pg/ml, and the lowest caspase-3 levels were found in the C1, with levels of 0.060 ± 0.006 pg/ml (Table 5). Caspase-3 levels in each group decreased at 48 hours of treatment compared to 24 hours (Table 6).

In addition to assessing caspase-3 levels, this study also assessed the level of apoptosis in HepG2 cells. The lowest apoptotic rate in the first 24 hours of treatment was in the C1 with an apoptotic rate of 3699.86 ± 1741.16, and the highest apoptotic rate was in the Cb with an average of 2763000.00 ± 961994.11. At 48 hours after treatment, the level of apoptosis in the C1 was still the group with the lowest rate, with an average of 1631028.57 ± 517758 (Table 7). The level of apoptosis at 48 hours of treatment decreased significantly compared to 24 hours with a p-value <0.05. Comparative tests showed that treatment groups C1, C2 and C3 had significantly lower apoptosis levels compared to the control group (Cb) after 24 hours and 48 hours of treatment (Table 8).

Table 5. Simultaneous Difference Test for Caspase-3 Variables Based on Treatment Preparations

Groups	Caspase 3	
	24 hours of treatment (Mean ± SD)	48 hours of treatment (Mean ± SD)
C1	0.071 ± 0.004	0.060 ± 0.006
C2	0.074 ± 0.005	0.068 ± 0.004
C3	0.075 ± 0.007	0.059 ± 0.003
Cb	0.071 ± 0.007	0.063 ± 0.005
p-value	0.458	0.008

Table 6. Partial Difference Test (Post Hoc) Caspase-3 Variables Based on Treatment Preparations

Groups	Caspase 3	
	24 hours of treatment (p-value)	48 hours of treatment (p-value)
Cb vs C1	<0.001	<0.001
Cb vs C2	<0.001	<0.001
Cb vs C3	<0.001	<0.001
C1 vs C2	0.455	0.009
C1 vs C3	0.960	0.687
C2 vs C3	0.486	0.024

Table 7. Simultaneous Difference Test for Apoptotic Variables Based on Treatment Preparations

Groups	Apoptosis Rate	
	24 hours of treatment (Mean ± SD)	48 hours of treatment (Mean ± SD)
C1	3699.86 ± 1741.16	4476.86 ± 750.17
C2	138942.86 ± 60091.73	034302.86 ± 11605.87
C3	19142.86 ± 4789.53	10510.86 ± 2291.25
Cb	2763000 ± 961994.11	1631028.57 ± 517758.77
p-value	<0.001	<0.001

Table 8. Partial Difference Test (Post Hoc) Apoptotic Variables Based on Treatment Preparations

Groups	Apoptosis Rate	
	24 hours of treatment (p-value)	48 Hour of treatment (p-value)
Cb vs C1	0.002	<0.001
Cb vs C2	0.002	<0.001
Cb vs C3	0.002	<0.001
C1 vs C2	0.002	0.831
C1 vs C3	0.002	0.966
C2 vs C3	0.002	0.865

Necrosis effect

At 24 hours after treatment, the lowest necrosis rate occurred in C2, with an average of 1.596 ± 0.134, and the highest necrosis rate was in C3 (15.078 ± 0.857). The necrosis rate in C2 was the lowest at 48 hours of treatment, with a necrosis rate of 2.066 ± 0.369, and the highest necrosis rate was in C3, with an average of 15.066 ± 0.931 (Table 9). Compared to the Cb, C2 significantly reduced the necrosis rate of HepG2 cells. In contrast, the C1 and C3 had a significantly increased necrosis rate compared to the control group (Table 10).

Proliferation effect

The lowest proliferation rate at 24 hours of treatment was in C1 at 0.187 ± 0.039, and the highest in C2 with an average proliferation rate of 0.777 ± 0.123. The lowest proliferation rate after 48 hours of treatment was in group C1 0.232 ± 0.075, while the lowest was in Cb (Table 11). There was a significant increase in the proliferation rate of each group at 48 hours of treatment compared to 24 hours of treatment with a p-value <0.05 (Table 12).

Table 9. Simultaneous Difference Test for Necrosis Variables Based on Treatment Preparations

Groups	Necrosis Rate	
	24 Hour of treatment (Mean ± SD)	**48 Hour of treatment (Mean ± SD)**
C1	11.184 ± 2.182	13.409 ± 1.016
C2	1.596 ± 0.134	2.066 ± 0.369
C3	15.078 ± 0.857	15.066 ± 0.931
Cb	9.035 ± 0.990	5.670 ± 1.513
p-value	<0.001	<0.001

Table 10. Partial Difference Test (Post Hoc) Necrosis Variables Based on Treatment Preparations

Groups	Apoptosis Rate	
	24 Hour of treatment (p-value)	**48 Hour of treatment (p-value)**
Cb vs C1	0.085	<0.001
Cb vs C2	0.002	<0.001
Cb vs C3	0.002	<0.001
C1 vs C2	0.002	<0.001
C1 vs C3	0.004	0.007
C2 vs C3	0.002	<0.001

Table 11. Simultaneous Difference Test for Proliferation Variables Based on Treatment Preparations

Groups	Necrosis Rate	
	24 hours of treatment (Mean ± SD)	**48 hours of treatment (Mean ± SD)**
C1	0.187 ± 0.039	0.232 ± 0.075
C2	0.777 ± 0.123	1.031 ± 0.180

C3	0.258 ± 0.040	0.290 ± 0.048
Cb	0.657 ± 0.196	1.084 ± 0.031
p-value	<0.001	<0.001

Table 12. Partial Difference Test (Post Hoc) Proliferation Variables Based on Treatment Preparations

Groups	Apoptosis Rate	
	24 Hour of treatment (p-value)	48 Hour of treatment (p-value)
Cb vs C1	0.002	0.002
Cb vs C2	0.338	0.337
Cb vs C3	0.002	0.002
C1 vs C2	0.002	0.002
C1 vs C3	0.013	0.085
C2 vs C3	0.002	0.002

DISCUSSION

MSCs generally have low immunogenicity due to low expression of class II MHC antigens and class I MHC molecules [18]. In addition, MSCs do not contain co-stimulatory molecules such as CD80, CD86 and CD40, which need recognition by immune cells [2]. According to this research, MSC did not express CD89, CD86 and CD40. UC-MSC showed the presence of three dominant markers: CD 73+, CD 90+ and CD 105+. MSCs have positive markers for CD73, CD90 and CD105 presence, indicating their ability to differentiate into adipocytes, osteoblasts and chondroblasts under certain conditions [19].

The International Society for Cellular Therapy (ISCT) applies several criteria to identify MSC, one of which is recognized through the expression of its surface antigen. To identify MSCs, the cell culture results must contain the expression of CD105, CD73 and CD90. To assess the purity of MSCs, the levels of the three surface expression antigens were > 95% [20].

MSC's therapeutic effect is produced not only by the differentiation of the cells but also through dissolved factors or conditioned medium from MSC [21]. MSC-CM is known to significantly inhibit apoptosis and stimulate the proliferation of hepatocyte cells in studies with animals subjected to acute liver failure [2,22]. Treatment with MSC in the liver of mice experiencing ischemia can increase the expression of regeneration proteins such as Vascular endothelial growth factor (VEGF), TNF-α, Hepatocyte growth factor (HGF) and several other molecules compared to the control group [23]. In our study, VEGF expression was expressed by the UC-MSC CM.

In this study, MSC had an effect by reducing NF-κB levels compared to controls. Increasing NF-κB levels lasted up to 48 hours after treatment. This condition was similar to the group that received silymarin, either silymarin alone or MSC with silymarin. These results are in line with research conducted by Lin et al., which showed that MSCs could produce nerve growth factor (NGF), which acts to inhibit NF-κB and reduce the expression of the anti-apoptotic gene B cell leukaemia-xl (Bcl-xl) [24]. MSCs also secrete HGF, inhibiting the NF-κB pathway and reducing fibrosis in LX2 cell lines [25]. Consistent with the results of this study, an in vivo study using mice as research subjects showed that MSC administration could reduce the occurrence of liver fibrosis in mice injected with Carbon tetrachloride (CCL4) by inhibiting and causing apoptosis of HSCs [26]. MSCs also have anti-inflammatory effects by releasing anti-inflammatory cytokines such as IL-4 and IL-10, which cause immune

cells to secrete anti-inflammatory cytokines [26]. MSCs inhibit the synthesis of proinflammatory cytokines such as TNF-α, IFN-γ and IL-17 in various immune cells, including T cells, natural killer cells, neutrophils and Kupffer cells [27]. Based on research, the silibin content found in silymarin reduces the expression of NF-κB signalling activity. Silybin effectively prevented hepatic steatosis, day fibrosis and inflammation in rats with methionine-choline deficiency. The underlying mechanism is related to changes in gene expression related to lipid metabolism, activation of the Nrf2 pathway and inhibition of the NF-kB signalling pathway in the liver [16].

This study showed that the group that received MSC therapy had significantly higher caspase-3 levels than the control group. However, when examining the apoptosis rate, it was shown that the control group had a higher rate than the treatment group. The lowest rate of apoptosis was found in treatment with silymarin. Caspases are categorized as proinflammatory cytokines and pro-apoptotic cytokines, depending on their involvement in cellular responses. Caspase-3 is a pro-death caspase that plays a central role in apoptosis [28]. Suppression of caspase-3 produces a protective effect against hepatocellular damage, cell death and proinflammatory signals. Decreased caspase can markedly reduce the occurrence of collagen deposition and the development of liver fibrosis [29]. The results of previous studies indicate that caspase-3 activation plays an essential role in the occurrence of steatohepatitis and fibrosis [29]. Another in vivo study showed that transplantation with pretreated MSCs increased albumin levels, cytokeratin 8, 18 and anti-apoptotic protein Bcl-xl. In contrast, pro-apoptotic proteins Bcl-xl and caspase-3 decreased [30]. Increased caspase-3 levels in the MSC-treated group could be due to increased MSCs' apoptosis.

Liver fibrosis will cause an increase in the induction of apoptosis of hepatocyte cells. It causes an increase in apoptotic bodies and reactive oxygen species (ROS), activating hepatic stellate cells (HSCs) and stimulating inflammatory cell infiltration [31]. MSCs secrete cytokines, chemokines, growth factors and anti-inflammatory factors, which are helpful for anti-apoptotic activity [32]. Hepatocyte apoptosis is one of the factors that can cause liver fibrosis. On examination of TUNEL staining on fibrosis-induced rat hepatocyte cells given MSC treatment, there was a significant reduction of hepatocyte apoptotic nuclei compared to the control without treatment [33]. Various studies of silymarin have shown that silymarin has anti-cancer properties through the regulation of apoptosis [15]. Silymarin has an anti-fibrosis effect on the liver; in this study, it was shown that the anti-fibrosis effect of silymarin is by reducing the occurrence of apoptosis of hepatocyte cells.

This study also showed that MSCs could reduce the necrosis rate compared to controls in HepG2 cells. Necrosis is a significant finding in patients with acute or chronic liver disease [34]. However, the opposite condition occurred in the treatment given with silymarin. There was an increase in the incidence of necrosis compared to controls. In an in vivo study on liver necrosis induced by toxic doses of paracetamol, the administration of silymarin did not make a significant difference compared to the control group regarding the incidence of necrosis observed under a microscope [17].

In addition to being anti-apoptotic, MSCs can stimulate the proliferation and regeneration of hepatocyte cells through the secretion of various growth factors and prostaglandin E [23]. Previous research data showed that not only does MSC cause hepatocyte proliferation, but secretomes from MSCs also significantly reduce apoptosis and cause a proliferation of hepatocyte cells in animal models of acute liver injury [2]. The proliferative effect of HepG2 cells after administration of MSC is in line with previous studies. Silymarin can also act as a cell development agent to induce cell cycle progress so that proliferation and liver regeneration occur. Silymarin can increase growth factors such as HGF, tumour growth factor alpha (TGF-α), and TGF-1 [35]. In this study, the administration of silymarin increased the proliferation rate but was lower than the control.

This study has several limitations, including the need for more specific examinations in assessing the various variables used. The proliferation assessment with the MTT assay can be supplemented with staining examinations, for example, with ki67 or BrdU. In addition, it is necessary to examine other markers related to immunomodulation, anti-apoptosis, necrosis, and proliferation to assess the pathways of the effect of MSC on liver fibrosis. This study is an initial study that can be used as a reference in future studies to assess the effect of

mesenchymal stem cells on the pathogenesis of liver fibrosis. This study can also be an essential reference for MSC with or without silymarin as a treatment for liver fibrosis.

CONCLUSION

Mesenchymal stem cells have the ability of immunomodulation, anti-apoptosis, anti-necrosis and increased proliferation of HepG2 cells in fibrosis conditions. Combining mesenchymal stem cells with silymarin did not provide better results than treatment with mesenchymal stem cells as monotherapy. Further research is needed to assess mesenchymal stem cells' effectiveness in managing liver fibrosis.

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CONFLICT OF INTEREST

The author declares there is no conflict of interest.

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