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Mini Review on Mesenchymal Stem Cell Differentiation into Hepatic Lineage and Herbal Induced Hepatoprotection

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Abstract
Liver transplantation is currently the most successful treatment for severe cirrhosis and acute liver failure. The shortage of donors, surgical difficulties, immunosuppression, and high cost of care are a few drawbacks of this technique. Stem cell transplantation is one of the alternatives to using a complete liver that has been proposed as an efficient alternative therapy for hepatic disorders. Preclinical and clinical research has assessed this treatment's potential. The immunomodulation, differentiation, and antifibrotic capabilities of mesenchymal stem cells are critical for liver regeneration, although the processes of mesenchymal stem cell transplantation are currently poorly understood. In the last decade, there has been substantial progress in the field of MSC-dependent liver regeneration and immunomodulation. We have outlined the therapeutic potential of MSCs in the management of cirrhosis and liver failure in this succinct review, highlighting their regenerative and immunomodulatory properties. Additionally, we highlighted the hepatoprotective properties of herbal/medicinal plants and their secondary metabolites (Phytochemicals). Conclusion: Effective and secure MSC-based treatment for acute and chronic liver failure continues to be a difficult problem that needs more study and ongoing collaboration between physicians, researchers, and patients.

Keywords: Mesenchymal stem cells, Adipose-derived mesenchymal stem cell, Drug-induced liver damage, Hepatoprotective herbal drugs.

Abbreviations:

AD-MSC- Adipose derived mesenchymal stem cell

AFP - Alpha fetoprotein

ALB- Albumin

Ang-1 - Angiopoietin-1

BEC-like cells - Biliary epithelial cells- like cells

BM-MSC- Bone marrow derived mesenchymal stem cell

CB-MSC - Cord blood derived mesenchymal stem cell

DILI - Drug-induced liver damage

DP-MSC- Dental pulp derived mesenchymal stem cell

EGF- epidermal growth factor

ERK- Extracellular signal-regulated kinase (ERK)

ESCs- Embryonic stem cells

FGF- Fibroblast growth factor

HBV- Hepatitis B virus

HGF- Hepatocyte growth factor

HLCs- Hepatocyte-like cells

H-MSC- Heart-derived derived mesenchymal stem cell

IDO- Indoleamine 2,3 dioxygenase

IGF- Insulin-like growth factor

iPSCs- Induced pluripotent stem cells

ISCT- International Society for Cellular Therapy

MAPK- Mitogen-activated protein kinase

MSCs - mesenchymal stem cells

PGE2- Prostaglandin E2

TSA- Trichostatin A

UC-MSC - Umbilical cord derived mesenchymal stem cell

VEGF- Vascular endothelial growth factor

WJ-MSC - Wharton’s jelly-derived derived mesenchymal stem cell

Background

Chronic liver disorders, which eventually proceed to fibrosis and cirrhosis, impact an estimated 1.5 billion individuals worldwide today (Moon et al., 2020). Liver transplantation is presently the only effective treatment for end-stage liver-related suffering patients as it significantly improves the prognosis. In recent years, immunosuppressive medicines and surgical techniques have made transplantation more effective, and the international organ transplant market is anticipated to grow significantly over the next decade (Lee et al., 2021). However, as of now, patients die before receiving transplantation as a result of the waiting period, hence other therapeutic techniques such as hepatocyte transplantation and bioartificial liver devices can be regarded as alternative (Yu et al., 2012). A source of functional hepatocyctes for transplantation can be obtained from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs). As an adjuvant or alternative therapy to liver transplantation, MSCs have a wide spectrum of therapeutic possibilities.

ESCs and iPSCs were considered to be extremely valuable candidates for disease regenerative therapy, particularly iPSCs with no or mild immunological rejection. These cells have the capacity to self-renew and differentiate into three germ layers derived lineages. However, the direct transplantation of ESCs/iPSCs to treat illness is severely constrained by their tumorigenicity. However, MSCs have been considered prospective candidates for regenerative treatment because of their potent immunomodulatory, anti-inflammatory, and proregenerative properties (Jung et al., 2012).

The first characteristic of MSCs is that they are pluripotent stem cells capable of transforming into hepatocyte-like cells both *in vivo* and *in vitro*. A second advantage of MSCs is easy accessibility as they can be obtained from a multitude of potential sources, including adipose tissue, synovial membranes, peripheral blood, umbilical cord blood, dermis, muscle, and liver. Crucially, the extracted MSCs retain their pluripotent potential, strong proliferative ability, and *ex vivo* growth potential. Thirdly, MSCs can move and engraft at wounded tissue locations. Fourth, MSCs exhibit immunosuppressive qualities, making allogeneic transplantation possible. MSCs have immunosuppressive properties as well as anti-fibrotic and antioxidant properties, which help protect the liver from fibrosis and oxidative damage. Lastly, MSCs also release extracellular vesicles to promote regeneration of impaired tissues like liver parenchyma by releasing growth factors and cytokines (Lee et al., 2021). In this review, we will focus on the *in vitro* differentiation of MSCs into hepatogenic lineage and phytopharamaceuticals that anticipates as more effective treatments for liver diseases.

MSC isolation and characterization

MSC sources

According to recent data, the final differentiation of MSCs is influenced by their origin and can influence tissue regeneration (Yun et al., 2014). MSCs are typically classified on their basis of derived source: Bone marrow MSCs (BM-MSCs), adipose derived (AD-MSCs), Dental pulp-MSCs (DP-MSCs), Heart-derived MSCs (H-MSCs), menstrual blood, neonatal sources such as the placenta, amnion, chorion, umbilical cord (UC-MSC) (Yang et al., 2021), and cord blood (CB), and Wharton's jelly (WJ)-derived MSCs (Afshari et al., 2021).

MSC characteristics

According to International Society for Cellular Therapy (ISCT), the characteristics of MSCs includes fibroblastic morphology; plastic adherence; differentiation to osteoblasts, adipocytes and chondroblasts; as well as positive expression of CD105, CD44, CD90, and CD73, with negative expression of CD45, CD34, CD14, or CD11b, CD79α, or CD19 and HLA-DR surface

markers (Dominici et al., 2006). Notably, MSCs from different sources may have distinct *in vitro* properties and surface molecular expressions (Orbay et al., 2012). *In vitro* research in this field is growing exponentially due to significant characteristics of MSCs. MSCs can be cultured using a simple isolation method (Si-Tayeb et al., 2010) whereas their potential differentiation capacity allows them to differentiate into chondrocytes, adipocytes, and osteoblasts under certain conditions. Additionally, as MSCs are non-immunogenic, they produce several cytokines, but do not express MHC class I and II antigens or only express low levels of these antigens and also lack B7 co-stimulatory molecules that are essential for initiating immune responses. As a result, they are an immune compatible universal source of stem cells for transplantation with no immunological rejection and hence no immunosuppression required (Orbay et al., 2012). Thus, the easy isolation procedures, high differentiation capability, and immunomodulatory features of MSCs make it a suitable stem cell source for regenerative medicine and hepatocyte transplantation, respectively.

Tissue specificity

The differentiation potential and the gene expression patterns of MSCs derived from different sources can differ significantly. CD36, CD163, CD271, CD200, CD273, CD274, CD146, CD248, and CD140b are non-classical markers that are being used to differentiate MSCs from distinct sources (Camilleri et al., 2016). Single-cell RNA sequencing study has recently found a population of highly proliferative multipotent progenitors marked by dipeptidyl peptidase-4/CD26 during the formation of subcutaneous adipose tissue in mice. A subpopulation of these progenitor cells is composed of CD142-positive and ICAM-1-positive committed preadipocytes (Merrick et al., 2019). Additionally, AD-MSCs show greater efficacy in supporting hematopoiesis and angiogenesis than BM-MSCs (Strioga et al., 2012).

MSC priming

MSCs can express a variety of immune regulatory factors, such as Nitric oxide, Prostaglandin E2 (PGE2), indoleamine 2,3 dioxygenase (IDO), IL-6, IL-10, and HLA-G, when they are delivered to a damaged liver. On contrary, MSCs may promote myofibroblast activity and aggravate hepatic fibrosis depending on the quantity of inflammatory cytokines such as TNF- α, IL-1 β and IFN- γ (di Bonzo et al., 2008; Yun et al., 2014). An *in vitro* priming process of MSCs can enhance therapy effectiveness and reduce pro-fibrotic characteristics. MSCs primed with IFN- γ express IDO, inhibiting T and NK cell activation (Krampera et al., 2006), leading to enhanced immunosuppression owing to a decreased susceptibility to NK-cell mediated killing (Giuliani et al., 2014). However, a combination of IFN- γ and TNF- α primed MSCs increased anti-inflammatory effects (Linero and Chaparro, 2014). A lack of cell survival after transplantation is another reason for low efficacy of MSC-based therapy, but priming has been found to improve cell survival after transplantation (Kang et al., 2020). According to recent studies, MSCs have been demonstrated to be more viable when treated with zeaxanthin dipalmitate as it suppresses inflammation, oxidative damage as well as apoptosis in ADMSCs (Liu et al., 2017). In order to determine whether the benefits of priming MSCs prior to transplantation are sustained long-term after differentiation, further investigation is required.

MSC Differentiation into Hepatocyte-Like Cells

MSCs with pluripotence are able to differentiate into endoderm, mesoderm, and ectoderm cells. There is a wide range of active molecules secreted by MSCs. These components include hematopoietic growth factors [including hepatocyte growth factor (HGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), epidermal growth factor (EGF)]; angiogenic growth factors [vascular endothelial growth factor (VEGF), vascular cell adhesion molecule 1,

angiopoietin-1 (Ang-1), leukemia inhibitory factor]; trophic molecules [oncostatin M, osteopontin], immunomodulatory cytokines [interleukin-7]; chemokines [CXCL12] and chemical compounds [dexamethasone, insulin-transferrin-selenium, retinoic acid, nicotinamide, norepinephrine, sodium butyrate, and dimethyl sulfoxide. Indeed, the ability of MSCs to differentiate into liver-specific hepatocyte-like cells in the presence of cytokines and growth factors, and has been demonstrated in both *in vivo* and *in vitro* experiments (Schwartz et al., 52009; Méndez-Ferrer et al., 2010; Madrigal et al., 2014).

MSCs can be stimulated to differentiate in culture, but the preferred route for differentiation into a specific cell type is in an organ-specific microenvironment (Sato et al., 2015).The expression of hepatocyte-specific genes distinguishes hepatic-differentiated cells, and these genes are affected by the microenvironment (Dai et al., 2009). Notably, liver function gets restored via mechanisms reminiscent of microenvironmental cues rather than cell fusion, within a week of transplantation (Jang et al., 2004). MSCs do not directly convert into functional hepatocytes, as Zhang et al. (2017) have showed; but rather differentiate into biliary epithelial cells (BEC)-like cells, which then differentiate into hepatocyte-like cells (HLCs). Other research, on the other hand, show that MSC trans-differentiation is uncommon after MSC infusion in animal models (Dai et al., 2009). According to these findings, MSCs exert their therapeutic benefits via both direct cell differentiation and indirect paracrine signaling.

Induction of ADMSC to Hepatocyte-Like Cells (HLCS)

Clinical uses of ADMSCs in regenerative medicine have a lot of potential. With minimally invasive methods such as liposuction, a significant amount of adipose tissue can be retrieved. Patients with liver illness may find that transplantation is a simpler, more effective, and safer alternative to whole organ transplantation for their treatment (Yamamoto et al., 2003). ADMSCs have many of the characteristics of BMSCs but, unlike BMSCs, are unable to differentiate into osteogenic and adipogenic cells. *In vitro* MSC development into hepatocyte-like cells has not been well studied. Co-culturing with Huh-7 cells was enough to promote hepatic differentiation (Yin et al., 2015).

Researchers have demonstrated that ADMSCs quickly develop into HLCs by altering their shape and function in response to cytokine treatment (Liang et al., 2009). ADMSCs can be induced to differentiate into hepatocytes by various methods. It is possible to induce the differentiation of ADMSCs into hepatocytes by culturing them in hepatogenic medium containing dexamethasone, insulin, HGF, and EGF for 2 weeks after which extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase-mediated (MAPK) signaling pathways are activated for the final stage of the hepatogenic differentiation process (Liang et al., 2009). In line with this, albumin (ALB), alpha-2-macroglobulin, complement protein C3, and selenoprotein P1, appear as early markers while CYP, apolipoprotein E, acyl-CoA synthetase long-chain family member 1, and angiotensin II receptor, type 1 reflect as late markers during stepping up of hepatogenic differentiation of MSCs or generation of HLCs. Finally, when THY1 and inhibitor of DNA binding 3 expression levels are low, these cells lack the stem cell characteristics (Bonora-Centelles et al., 2009). There are several liver-specific functions performed by HLCs derived from ADMSCs, including glycogen synthesis, urea production, ALB secretion, the uptake of low-density lipoproteins, CYP enzyme activity, manifestation of carbamoyl phosphate synthetase 1 as well as perivenous functions such as expression of CYP450 subtype 3a11 and CD26 (Banas et al., 2007; Aurich et al., 2009). Moreover, HLCs and liver tissue have striking similarities in their gene expression profiles, gene clusters, genes, and particularly in signaling pathways and MET transitions (Yamamoto et al., 2008).

Despite the fact that current studies use a variety of differentiation processes, ADMSC-derived HLCs exhibit immature hepatocyte activities, prompting researchers to seek out novel ways to improve HLC functions. In an *in vitro* investigation, Jiangyang et al. (2013) found that serum from rats with a 70% partial hepatectomy enhanced hepatogenic differentiation of ADMSCs by upregulating IL-6 and HGF production. In line with this, after incubation with liver extract, ADMSCs showed more rapid changes in cellular shape and produced higher levels of alpha fetoprotein (AFP) and ALB than after culture in the presence of substances such as hepatocyte growth factor, fibroblast growth factor, and oncostatin M (Nhung et al., 2015). Furthermore, Trichostatin A (TSA), a hormone, aids in the subsequent differentiation of human MSCs towards endodermal lineages. TSA concentrations over a certain threshold can stop the cell cycle, which results in cell death and differentiation. Despite increases in ALB mRNA and protein levels, as well as their ability to store glycogen, the hepatocyte-like cells produced from ADMSCs in the current study performed far better when treated with TSA. The alleviation of liver damage after treatment with ADMSCs highlighted their potential as a novel therapeutic strategy for liver diseases or injury. The work of Yin et al. (2015) revealed that TSA is required for the differentiation of human MSC into functional hepatocyte-like cells. Alizadeh et al. (2016) reported that TSA, enhanced the hepatogenesis of ADMSCs by enhancing miR-122, ALB, HNF4*, HNF6 expression, and downregulates the AFP level. Furthermore, ADMSCs differentiated into HLCs more rapidly in the presence of dimethyl sulfoxide, a cryoprotectant, is evidenced by changes in cell morphology, expression of ALB, HNF4, and HNF6 and larger glycogen stores. Nevertheless, an experimental study has shown that ADMSCs acquire the functional properties of primary human hepatocytes *in vitro* after incubated for three days with activin A and FGF4 and then incubated for 10 days with HGF, FGF1, FGF4, dexamethasone, insulin-transferrin-selenium, dimethyl sulfoxide, and nicotinamide (Banas et al., 2009). Strikingly, Xu et al. (2015) readily transformed ADMSCs into functional hepatocytes within 9 days, using a three-step protocol that involved incubation with IDE1, CHIR99021, then with FGF4, and HGF, and final exposure to HGF, EGF, oncostatin M, dexamethasone, and insulin–transferrin–selenium.

ADMSC differentiation can also be facilitated by gene modification, in addition to hepatic medium culture. The use of microRNAs for genetically modified expression of growth factors (GFs) and cytokines is a viable method for differentiating ADMSC into HLCs. Several studies have shown that miR-122 and miR-27b play a critical role in the differentiation of ADMSCs into hepatocytes (Davoodian et al., 2014; Chen et al., 2014). Hepatogenic ADMSCs overexpressing OCT4 and SOX2 showed no changes in MSC markers or morphology, but their expression of ALB, urea, and glycogen was enhanced (Han et al., 2015). Thus, human ADMSCs have the potential to be a helpful source of hepatocyte regeneration and a viable alternative to liver transplantation.

MSCs Derived Exosomes and Its Role

As a promising cell-free method for liver regeneration, MSCs-exosomes have attracted the attention of several researchers. Concerns with the direct use of MSC, including as immunogenicity and tumor formation, have been addressed. MSCs can secrete exosomes to maintain cell and tissue homeostasis. Exosomes may carry a range of tiny biomolecules, such as mRNAs, miRNAs, single-stranded DNA, double-stranded DNA, mitochondrial-DNA and proteins to neighboring cells while shielding their contents from deterioration within a lipid bilayer encapsulation. Enzymes, transcription factors, lipids, and ECM proteins are among the

proteins with high cytoplasmic and membrane protein frequencies found in exosomes. Exosomes can be transferred to various cells to carry out particular jobs and range in size from 30 to 150 nm. Exosomes have antioxidant properties and induce downstream signaling in target cells. Apoptosis and inflammation are also suppressed by the genetic material they transmit to the target cells (Shokravi et al., 2022).

MSC-derived exosomes have a wide spectrum of therapeutic benefits and can mediate tissue healing, immunological modulation, and inflammatory control as natural vesicles ideal for gene transfer. Recent research has also shown that MSC-derived exosomes can mediate therapeutic effects in animal illness models, building on earlier studies that showed the clinical value of such exosomes in the treatment of bone fracture, cutaneous wounds, myocardial infarction, and acute hepatic damage (Nie et al., 2020). According to research by (Blazquez et al., 2014), ADMSC exosomes can reduce the accumulation of inflammatory cells and decrease T cell proliferation, differentiation, and activation. Donor MSC mitochondria can be transferred by exosomes to neighboring macrophages in order to boost oxidative phosphorylation and create an anti-inflammatory and highly phagocytic macrophage phenotype. ADMSCs induced the anti-inflammatory M2 phenotype in macrophages by delivering exosomes containing activated STAT3 (Morrison et al., 2017). Exosomes have the potential to control MSC development and migration in a targeted manner, providing a way to encourage tissue regeneration without using any cells. Thus, these genetically altered MSC-derived exosomes can be able to mediate tissue regeneration advantages, making them a promising new paradigm for cell-free MSC-based therapies.

Growth factors (GFs) and MSCs

A range of cell types can be formed from readily isolated MSCs by orchestrating and enhancing proximal or distal cell functionality via paracrine signaling and endocrine mechanisms for reconstructing damaged tissues and organs (Grimm et al., 2018). According to *in vivo* studies, paracrine secretion of GF by MSCs is a key mechanism of target tissue healing, as MSCs can migrate to sites of injury, yet the cells derived from them contribute only a limited amount of therapeutic effects. According to recent studies, MSCs mediate their therapeutic efficacy by secreting GFs and other bioactive molecules as these compounds stimulate angiogenesis (Fig. 1) (Shafei et al., 2017; Hu et al., 2018), regulate immune responses and inhibit apoptotic cell death and fibrosis (Nie et al., 2020).

GFs are well-known mediators that can help MSCs survive and proliferate, as well as being important drivers of tissue regeneration. Many researchers in the field of regenerative medicine have been investigating the use of MSCs to deliver specific GFs to a target region of tissue repair and reconstruction, either by employing cells naturally secreting these factors or by engineering these cells to overexpress GFs of interest. However, non-engineered MSCs have limited therapeutic efficacy due to low survival and GF secretion following implantation. In order to improve MSC survival and differentiation, researchers have looked into using MSCs that have been genetically engineered to express exogenous genes that can improve their ability to promote angiogenesis and target tissue homing (Shafei et al., 2017; Li et al., 2019). In addition to their ability to differentiate into particular cell types, these gene-modified MSCs also play a key role in inhibiting fibrosis, inflammation, and angiogenesis by secreting multifactorial GFs. Consequently, it is possible to improve both MSC engraftment and functionality and deliver therapeutic gene products targeting local tissue healing with these genetically engineered MSCs. Thus, the use of engineered MSCs to overexpress GFs seems to be an optimal method for improving their therapeutic efficacy (Nie et al., 2020).

Recent studies have shown that MSCs treated with GFs, such as HGF, FGF-2, PDGF-B, TGF-1, and VEGF-A, produce and secrete more GFs, which enhance the ability of MSCs to regenerate (Fierro et al,. 2011; Wakisaka et al., 2019). In an effort to increase the therapeutic potential of MSCs, some researchers have manipulated them to express many synergistic genes. IGF-1, for instance, is a growth factor that encourages cell survival, (Scioli et al., 2014) but HGF encourages angiogenesis while repressing fibrosis and inflammation (Wang et al., 2018). In a rat model system, radiation-induced hepatic damage can also be reduced and radiation-induced hematopoietic damage can be prevented by adenoviral-mediated overexpression of HGF (Li et al., 2014). Previous studies have shown that overexpression of these GFs enhances MSC-mediated tissue regeneration; making such GF overexpression strategies a key therapeutic target (Nie et al., 2020).

Drug Insult and Hepatic Lineage

Drug-induced liver damage (DILI) encompasses a wide range of liver symptoms. Nevertheless, hepatocyte death after drug consumption is the most frequent symptom. Acetaminophen toxicity is a famous illustration of how DILI can be predicted and it is a dose dependent. Low incidences of idiosyncratic DILI indicate that environmental and genetic factors may affect an individual's vulnerability to the insult mediated drugs. Drugs and their reactive metabolites often cause biochemical stress by binding to mitochondria or directly damaging them, which leads to oxidative stress, activation of stress signaling pathways, mitochondrial dysfunction, and endoplasmic reticulum stress. The activation of Cytochrome P450, lipid peroxidation, induction of nitric acid synthase, mitochondrial dysfunction, activation of pro-inflammatory mediators, and bile acid-induced liver cell death are only a few of the pathways that can cause hepatic diseases or toxicity (Bedi et al., 2016). Hepatocytes may also become susceptible to the lethal effects of immune responses due to drug-induced biochemical stress. A variety of adaptive mechanisms contribute to drug-induced injury mitigation, including antioxidant signaling (such as *Nrf2* signaling), mitophagy and autophagy, unfolded protein responses, anti-inflammatory responses, and immune tolerance (Yuan and Kaplowitz, 2013).

New research suggests that functional hepatocyte-like cells produced from MSCs are a better alternative for studying drug metabolism and toxicity. Liver injury is typically brought on by acetaminophen overdose and excessive alcohol intake. Perera et al. (2022) evaluated the toxic effects of APAP and ethanol on MSC-derived hepatocyte-like cells to that of HepG2 cells. The MTT assay demonstrated that after exposure to ethanol and APAP, cell viability declined in a concentration-dependent way in both types of hepatocytes.

In viral hepatitis, the immune system is activated by the hepatic virus, resulting in inflammation and liver damage. A significant necroinflammation of the liver occurs in chronic hepatitis as a result of an ongoing, ineffective immune response to hepatitis B virus (HBV). It has been noted in numerous models of chronic HBV infection that the liver damage is thought to be related to the activation of HBV-specific T lymphocytes. Virus clearance may potentially be hampered by cytokine abnormalities. Persistent HBV infections that lead to more severe liver damage is associated with T helper type cytokines such IL-4 and IL-10. Additionally, viral component HBx exaggerates the progression of chronic HBV (Lim et al., 2020). The ability of HBx antigen to decrease CD8+ T cell response by lowering IFN- production and initiating an apoptotic programme in CD8+ T cells was initially demonstrated by Lee et al. (2010). According to other research (Mahé et al., 1991; Lara-Pezzi et al., 1998; Lee et al., 2010), HBx generates innate pro-inflammatory IL-6, IL-8, and TNF- although frequently not at the level necessary for viral clearance in chronic HBV. It has been demonstrated, in particular, that IL-6 triggers the

transition from acute to chronic inflammation by drawing monocytes to the sites of inflammation (Gabay, 2006).

Hepatoprotective Herbal Drugs

Since ancient times, medicinal plants found in nature have served as a huge repository of cures for a variety of diseases. Herbs have a wide range of pharmacological properties because of the numerous phytoconstituents they contain. Around the world, 80 percent of people rely in some way on herbal remedies for their basic healthcare (Fig 1). Herbal medications have surpassed conventional drugs in popularity due to their decreased risk of adverse effects, efficacy with chronic illnesses, lower cost, and broad availability (Bedi et al., 2016). There are several contemporary medications or therapies available for the treatment of hepatic problems, but there is still a need for novel drug discovery that can target numerous disease pathways. Currently, medical treatments have been extensively based on natural products. There is a wealth of information in both ancient and modern science that supports the use of traditional remedies to treat many serious illnesses with few or no side effects. There are numerous herbal remedies that have been scientifically proven to be hepatoprotective, such as *Silybum marianum*, *Picrrorhiza kurroa, Andrographis paniculata, Phyllanthus niruri,* and *Aerva lanata* etc., that are frequently used to treat liver problems. Table 1 represents some of the herbal phytochemicals that are used for the liver diseases in *in-vitro* studies.

Picrorhiza kurroa

Picrorhiza kurroa Royle ex Benth (family: *Scrophulariaceae*) is a familiar herb in traditional (Ayurvedic and Unani) medicine under the name Kutki or Kutaki. The primary active components of *Picrorhiza kurroa* Royle ex. Benth's roots and rhizomes are picrosides I, II, and apocynin (Kutki). Kutkin, the bitter component of this plant, is a combination of two iridoid glycosides, picroside I and picroside II. Iridoid glycosides, picrosides, kutkins, apocynin, and cucurbitacin glycosides are some of the main bioactive constituents contained in roots and rhizomes of the plant. *P. kurroa* has been found to have anti-inflammatory properties, immunomodulatory properties, and antioxidant functions. In Ayurveda, the plant has long been used ethnopharmacologically to treat dyspepsia, hepatic and upper respiratory tract infection, fever, and scorpion sting. Previous research revealed that *P. kurroa* has hepatoprotective effects against aflatoxin, carbon tetrachloride, and amanita poisoning (Zahiruddin et al., 2017).

Silymarin maranium

For millennia, *Silybum marianum* (milk thistle) has been used safely as a natural herbal therapy to treat liver and gallbladder diseases, including hepatitis, cirrhosis and jaundice. Additionally, new effects of silymarin have been discovered over time in conditions such non-alcoholic fatty liver disease, alcoholic liver disease, viral hepatitis, hepatocellular carcinoma, and iatrogenic liver disease (Abenavoli et al., 2011; Camini and Costa, 2020). In most cases of liver disease, including cirrhosis and liver damage from alcohol abuse, silymarin has been shown to be beneficial as a supportive treatment. It is a complex mixture of flavonolignans, flavonoids (taxifolin, quercetin) and polyphenols derived from plants. Furthermore, Eurosil 85 is a patented formulation designed to improve silymarin oral bioavailability (Gillessen and Schmidt, 2020).

Silymarin has been used as a hepatoprotective agent for many years in popular culture and basic research. There are basic mechanisms by which it exerts the therapeutic effects. It controls the intracellular glutathione concentration and functions as an antioxidant by removing reactive species. The nuclear-kappa B transcription factor (NF-kB) is inhibited, and the release of cytokines is suppressed. It functions as a cell membrane stabilizer and permeability regulator,

preventing hepatotoxic substances from entering hepatocytes. Additionally, it stimulates ribosomal RNA synthesis, which can accelerate hepatic regeneration and inhibits the transformation of stellate hepatocytes into myofibroblasts (Camini and Costa, 2020).

According to preclinical evidence, silymarin can lessen oxidative stress and its associated cytotoxicity, protecting liver cells that are still healthy or that have not yet sustained irreparable damage. The use of silymarin significantly decreased the number of liver-related mortality in clinical trials including cirrhotic patients (Gillessen and Schmidt, 2020). The antioxidant activity of silymarin is thought to be the mechanism by which it exerts these therapeutic effects. An antioxidant effect is exerted by scavenging the free radicals that cause lipid peroxidation and by affecting the enzyme systems linked to the cellular damage that causes fibrosis and cirrhosis. Furthermore, in model mice with alcoholic liver disease, Song et al. (2006) showed that silymarin (200 mg/kg), which was produced by giving ethanol through a gavage at a dosage of 5 g/kg bodyweight every 12 h for a total of three doses, decreased oxidative stress. The use of silymarin also averted declines in glutathione and lipid peroxidation and elevations in alanine aminotransferase and TNF- levels. Zaidi and Mahboob (2017) examined the impact of silymarin supplementation on various cirrhotic rat parameters and discovered that silymarin decreased total bilirubin and alanine aminotransferase activity while reestablishing electrolyte balance. Additionally, silymarin supplementation elevated levels of the antioxidant enzymes superoxide dismutase and catalase and decreased levels of the oxidative stress biomarker malondialdehyde, suggesting that silymarin gradually reduces thioacetamide-induced liver cirrhosis. In contrast, Abenavoli et al. (2018) examined a number of clinical studies in which silymarin was administered orally to patients at various doses and trial durations and discovered that there was insufficient high-quality evidence, leading to inconsistent data. However, hepatoprotective silymarin therapy should be initiated as soon as feasible in patients with fatty liver disease or DILI to fully benefit the liver and remove oxidative stress, the underlying cause of cytotoxicity.

Numerous studies have demonstrated that silymarin therapy may reduce liver damage in animals given acetaminophen (Vargas-Mendoza et al., 2014; Freitag et al., 2015). Silymarin was shown to protect mice from acetaminophen-induced hepatotoxicity by Kim et al. (2018) and Papackova et al, (2018). Kim et al. (2018) hypothesised that the glutathione conjugation ability plays a vital role in this detoxification since hepatic glutathione depletion is significantly reduced in acetaminophen-induced hepatotoxicity. According to Papackova et al. (2018) silymarin changed a number of parameters and decreased superoxide production, peroxynitrite levels, and oxidized glutathione levels, reducing oxidative and nitrosative stress. Its application in treatment is limited because well-structured trials are needed to collect scientific data and standardize the techniques for assessing therapeutic efficacy.

Andrographis paniculata

Andrographis paniculata (Malay: Hempedu Bumi) is native to Southeast Asia, specifically China, India, and Sri Lanka. This herb has long been utilised in Ayurvedic treatment, which is the traditional form of medicine used in India. It is most frequently used to cure and prevent infectious disorders as it strengthens the immunity. *A. paniculata* also exhibits analgesic, antioxidant, antibiofilm, gastroprotective, wound-healing, antiflavogenic, antimicrobial, anticancer, and antimalarial effects. The extract from *A. paniculata* is also thought to have a high potential for use as an anti-inflammatory medication with milder and fewer side effects, in addition to its herbal tonic effect on human health. Diterpenoids and 29-oxygenated flavonoids, such as 14-deoxy-11, 12-didehydroandrographolide, 12-difluco-lide, isoandrographicolide, andrographyrospermum, and stigmasterol, are abundant in *A. paniculata*. Andrographolide is the

primary bioactive phytochemical of *A. paniculata*, with a concentration of 0.2% found in the leaves. It is quickly absorbed, and P-glycoprotein has been implicated in intestinal absorption (Bardi et al., 2014).

The beneficial effects of *A. paniculata* for liver protection have also been scientifically proven on animal and human subjects in vitro and *in vivo*. In animals induced with alcohol, *A. paniculata* extract could repair hepatic injury, restore cellular permeability, and prevent enzyme leakage into the blood circulation. At a concentration of 50 mg/kg body weight of albino Wistar rat, the plant extract could restore antioxidative enzymes for liver protection. Treatment with *A. paniculata* extract could reduce Thiobarbituric acid reactive substances and lipid peroxides in the liver by up to 33–48%. Water extract was found to have more flavonoids but less phenolic compounds than ethanolic extract. In a rat model of liver inflammation caused by CCl4, an extract of the plant *A. paniculata* was needed at a dose of 100mg/kg body weight to reduce fatty accumulation. The extract also significantly reduced fatty degeneration and necrosis. The synthetic drug silymarin, on the other hand, required 100 mg/kg weight loss to reduce fatty tissue accumulation in a rat's liver. Nevertheless, in order to ensure the safety and effectiveness of herbal medication, the only restriction is the lack of scientific evidence supporting standardized herbal formulations (Chua, 2014).

Phyllanthus niruri

Phyllantus herbs have traditionally been used to treat liver illness. In the world's tropical and subtropical climates, the *Phyllanthus* genus has more than 600 species. *P. niruri* is one of the herbal plants from the family of Euphorbiaceae and the extract from this plant was widely used in the preparation of various ayurvedic formulations (Barros et al., 2006). South East Asian plant *P. niruri* has been used traditionally to treat a wide range of pathological conditions, including kidney stones, gallstones, hepatitis, jaundice, dyspepsia, bronchitis, influenza, asthma, dysentery, tumours, diabetes, vaginitis, and tuberculosis, as well as kidney and gallstone disorders. The analysis of its extract revealed several bioactive molecules and chemical agents including phyllanthin, hypophyllanthin, phyltetralin, niranthin, nirtetralin, hinokinin, lignans and isolintetralin (Calixto et al., 1998; Murugaiyah et al., 2009). Application of the extract exhibited antiulcer, antitumor and anticarcinogenic, hypolipidemic, antiviral, and antioxidant effects. In earlier investigations, the extract was used in a multiherbal remedy to treat liver problems. Numerous experimental investigations have shown that Phyllantus plants have hepatoprotective properties in both *in vitro* and *in vivo* settings. In-depth studies on *P. niruri's* hepatoprotective, antioxidant, antihyperuricemic, and lipid-lowering actions have been conducted. Recent research indicated that *P. niruri* exhibited hepatoprotective qualities against induced hepatitis in rats. The powerful antioxidant activities are owing to its abundance in flavonoids and phenolic chemical, which may also have significant effects on hepatoprotective activity (Al Zarzour et al., 2017). *P. niruri* isolates exhibited a potent hepatoprotective activity against CCl_4-induced hepatotoxicity in clone-9 and Hepg2 cell lines through reduction of lipid peroxidation and maintaining glutathione in its reduced form. This is attributable to their phenolic nature and hence antioxidative potential (Ezzat et al., 2020). Recent studies on the extract's function have demonstrated its effectiveness in protecting against viral hepatitis and toxicity brought on by various medicines or environmental toxins (Bhattacharjee and Sil, 2004). According to Amin et al., (2004) *P. niruri* extract protects rats from liver cirrhosis caused by thioacetamide. The liver of the rats exposed to hepatotoxic substances showed necrosis, lymphocyte infiltration in the centrilobular area, and growth of fibrous connective tissue. However, there was comparably little

inflammation and normal lobular architecture in the treated rats. These measures were substantially restored to normal levels by silymarin and *P. niruri* therapy.

Aerva lanata

A perennial prostrate or succulent undershrub with a height of 900 metres above sea level, *Aerva lanata* (Linn.) Juss. ex Schult. is a member of the *Amaranthaceae* family. It is often known as Gorakhabooti in Hindi or Mountain knotgrass in English and is a gift from nature owing to its multiple curative properties. It contains a wide variety of phytochemicals, including canthin-6-one and alpha-carboline alkaloids, flavonoids, phenolic acids, saponins, steroids, terpenoids, p-hydroxybenzoic acid, vanillic acid, syringic acid, and several other phytoconstituents, all of which contribute to its broad spectrum of pharmacological actions. It is a treasure of nature due to its antiurolithiatic, diuretic, hepatoprotective, anticancer, immunomodulatory, antioxidant, antibacterial, and countless other pharmacological properties (Mandal and Madan, 2015). A few of the health advantages of the *Aerva lanata* are hepatoprotection, nephroprotection, antiasthmatic, and antiamoebic effects.

Pharmacological tests were performed on mouse models using petroleum ether extracts, methanolic extracts, and isolated substances from *B. diffusa* and *A. lanata*. In albino rats, every extract tested exhibited hepatoprotective action. After the last doses of CCl4, animals treated with dosages of 100, 200, and 300 mg/kg had considerable liver histology and serum test protection (Ramachandra et al., 2011). A biherbal extract made from the leaves of *Aerva lanata* and Achyranthes aspera was tested in albino rats for its hepatoprotective properties against paracetamol-induced liver injury. At dosages of 200 and 400 mg/kg, serum glomerular enzymes, glutamic oxaloacetic transaminase, and alkaline phosphatase all reduced. In addition, mice treated with the extract had less liver weight (Anantha et al., 2012). In Sprague Dawley rats, the petroleum ether fraction of the whole plant was examined for its ability to hepatoprotect against liver damage brought on by carbon tetrachloride. The fraction at doses of 50 and 100 mg/kg body weight reduced histopathological alterations, corrected high activities of hepatic marker enzymes, and raised the activities of antioxidant enzymes. Additionally, there were improvements in the albumin/globulin (A/G) ratio, total protein in blood, and hepatic lipid peroxidation (Govindan and Vijayammal,, 2005). Furthermore, acetaminophen intoxication can be efficiently treated by mixing NAC with *Aerva lanata* hydroalcoholic root extract. Paracetamol-induced liver damage in rats was evaluated against the hydroalcoholic extract by Jaswanth et al. (2008). A dose of 600 mg/kg protected the liver, as shown by the decrease in alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase and bilirubin blood levels.

Conclusion and future prospects

MSCs are capable of moving to wounded liver tissues, transforming into hepatocytes, reducing hepatic inflammatory reactions and fibrosis, and displaying antioxidant characteristics. There are presently or have been more than 80 clinical trials employing MSCs to treat liver disease. Although the MSCs have demonstrated the ability to regenerate, their therapeutic potential for regeneration is limited. However, it seems that the majority of the therapeutic advantages of MSCs come from their ability to produce a range of chemicals, including cytokines, growth factors, and miRNAs, which support liver regeneration. Over the past 20 years, a number of clinical trials have examined the efficacy of MSCs therapy for conditions connected to the liver, but the outcomes have been inconsistent. The protein, DNA, and RNA produced by MSCs must be identified in order to determine the mechanism behind MSC therapy. Proteomics and transcriptomics can play a significant role in the analysis of the underlying process. Notably,

increasing the frequency of cells homing to the wounded liver is the key to magnifying the therapeutic benefits of MSCs. It is essential to look into the homing traits of MSCs in order to boost the therapeutic amount of these cells. Herbal products have long been used in traditional folk medicine to maintain health or to provide remedies for various human diseases. Liver disorders, including liver cirrhosis, benefit from therapeutic strategies employing compounds extracted from plants and herbs. High-efficiency liver protection medications can be made from natural ingredients. Ayurveda, Siddha, and Unani, three historic Indian medicinal systems, are the source of the bulk of drugs currently available. Many medicinal plants have been utilized effectively in preclinical and human investigations, including *Silybum marianum*, *Picrrorhiza kurroa*, *Andrographis paniculata*, *Phyllanthus niruri*, and *Aerva lanata*. The herbal medication has to be standardized in accordance with the bioactive components in a clear composition. The formulation must additionally specify a specific liver condition or stage of liver damage.

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Conflict of Interest
All the authors hereby declare that they do not have any potential conflicts of interest.

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This article does not contain any studies with human participants or animals performed by any of the authors.

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The manuscript draft is prepared by ST. Primary corrections and grammatically revised this manuscript by NJ. Author RR and NJ help to collection and analysis of data as well as revised manuscript.

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Table 1: *In-vitro* studies implicating hepatoprotective herbal plants and phytochemicals.

Herbal plants	Potential agents	Biological effects	Mechanism of action	Reference
Silymarin	Flavonolignans	Down-regulates the HCV core mRNA and protein expression	Antioxidant and anti-inflammatory	Bonifaz et al., 2009
		Blocks HCV entry and transmission by inhibiting microsomal triglyceride transfer protein activity, apolipoprotein B secretion, and infectious virion production into culture supernatants		Wagoner et al., 2010
		Inhibit CYP2C8 activity		Albassam et al., 2017
		Inhibiting inflammatory activity and metastases of HCC cells by down-regulating the mitochondrial transmembrane potential of liver cells.		Ramakrishnan et al., 2009
Phyllanthus niruri L	Flavanoids	Inhibits HBsAg secretion and HBsAg mRNA expression by up-regulation of annexin A7	-	Lam et al., 2006
Curcuma longa L	Curcumin	Inhibits cell proliferation and induces apoptosis on human liver carcinoma cells.	Pro-apoptotic	Notarbartolo et al., 2005
Camptotheca acuminata Decne	Camptothecin	Suppress SMMC-7721 cell growth by arresting cell cycle at the S and G2/M phases, and inducing mitochondrial pathway mediated apoptosis	Pro-apoptotic	Li et al., 2011

Coptidis rhizoma	-	Suppress HCC cells migration through Rho/ROCK signaling pathway inhibition		Wang et al., 2010
Brucea javanica (L.) Merr	-	Induces liver cancer cell apoptosis by regulating the mitochondrial dependent pathway and activating caspase 3	Pro-apoptotic	Lau et al., 2005
Curcuma aromatica	Beta-elemene	Induces cell apoptosis and suppress the proliferation of HepG2 cells by suppressing microtubular polymerization and decreasing alpha-tubulin.	Pro-apoptotic	Mao et al., 2013
Scutellaria baicalensis Georgi	Wogonin	Suppress the VEGF-C-induced lymphangiogenesis by a decrease in VEGF-C-induced VEGFR-3 phosphorylation through suppressing of IL-1beta and COX-2 production	Immunomodulatory	Kimura and Sumiyoshi, 2013
Bupleurum chinense DC	Saikosaponin D	Increases cell apoptosis by activation of caspases 3 and 7 and finally causing the DNA fragmentation	Pro-apoptotic	Wang et al., 2014
Coptis chinensis Franch	Berberine	Induces mitochondrial apoptosis in liver cancer cells and activating autophagic cell death in liver cancer cells by activation of Beclin-1 and suppressing the mTOR-signaling pathway.	Pro-autophagic and pro-apoptotic	Wang et al., 2010

Figure Ligand

Fig. 1 Overview on Mesenchymal Stem Cell therapy