Development of new approaches to treat patients with hepatic diseases that can eliminate the need for liver transplantation is imperative. Use of cell therapy as a means of repopulating the liver has several advantages over whole-organ transplantation because it would be less invasive, less immunogenic. Bone marrow-derived stem cells, such as hematopoietic, mesenchymal and endothelial progenitor cells are ideal candidates for liver regenerative therapies ***(Almeida-Porada et al., 2010)***.

Vitamin C (ascorbic acid) is a well known powerful antioxidant that plays a key role in biosynthesis of collagen and other ECM (extra cellular matrix) component including collagen type 1, beta 1-integrin and fibronectin ***(Prockop and Kivirikko, 1984),*** due to its role as a cofactor for proline hydroxylase and lysine hydroxylase which are enzymes involved in the hydroxylation of collagen. ECM is responsible for transmitting a wealth of chemical and mechanical signals that mediates key aspects of cellular physiology such as cell adhesion migration proliferation differentiation and death, thus preservation and regeneration of ECM are helpful for tissue regeneration ***(Nelson and Bissell, 2006).***

Recent studies show that vitamin C causes widespread, consistent, and remarkably specific DNA demethylation of 1847 genes in human embryonic stem cells (hESCs), including important stem cell genes, with clear demethylation at CpG

island boundaries, displays concomitant gene expression changes not only during differentiaition of hESCs but also during reprogramming of fibroblasts to induced pluripotent stem cell (iPSCs) ***(Chung et al., 2010)***.

It up regulates expression of pluripotency markers in (hESCs), such as Nanog, Oct4 & Sox2 genes ***(Potdar and D'Souza, 2010).***

Moreover, vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity ***(Wei et al., 2012)***. Telomerase is a ribonucleoprotein with reverse transcriptase activity, plays a pivotal role in maintaining telomere length and chromosomal stability in proliferating cells. In cells lacking telomerase activity, replication-associated telomere shortening limits the replicative lifespan. Therefore, in the context of liver regeneration, telomerase activation might be a cellular mechanism to confer an extended lifespan to replicating hepatocytes and hepatic progenitor cells ***(Wege and Brümmendorf, 2007)***.

The promotor of human telomerase reverse transcriptase is activated during liver regeneration and hepatocyte proliferation, hTERT human telomerase reverse transcriptase is induced in hepatocytes during liver regeneration, indicating a functional role for telomerase in human liver ***(Aldridge et al., 2012)***.

AIM OF THE WORK

Studying the effect of vitamin C supplementation on labeled bone marrow mesenchymal stem cell in CCl_4 induced injured liver by:

1. Assessment of vitamin C effect on stem cell ability to regenerate functioning hepatocytes through comparative measuring of liver functions (serum ALT, AST, and serum albumin levels).
2. Evaluate the antioxidant effect of vitamin C & stem cell by measuring malondialdehyde (MDA) levels in liver tissue.
3. Assessment of gene expression to the followings (albumin & matrix metalloproteinase -2 {MMP-2} genes) using real time PCR of studied hepatic tissue.
4. Histopathological examinations.

VITAMIN C (ASCORBIC ACID)

Vitamin C is a water-soluble vitamin, required for multiple biological functions as; it is a cofactor for several enzymes participating in the post-translational hydroxylation of collagen, in the biosynthesis of carnitine, in the conversion of dopamine to norepinephrine, in peptide amidation & in tyrosine metabolism. Besides, it has a role as an iron up-take regulator. In addition, vitamin C is a potent reducing agent and scavenger of free radicals in biological system ***(Aysun, 2009).***

Most animals are able to synthesize vitamin C from glucose, but humans, other primate lack (gulconolactone oxidase) the enzyme required for vitamin C synthesis so the presence of the vitamin in their diet is mandatory. Prolonged deprivation of vitamin C generates defects in the post-translational modification of collagen & ECM (extra cellular matrix) that cause scurvy and eventually death ***(Aysun, 2009).***

Vitamin C mega-dosage:

RDA (recommended dietary allowance) of vitamin C starts from 40 mg/day in infants up to 120mg/day in lactating women ***(Institute of Medicine, 2000).***

Linus Pauling, (1901-1994), a winner of two unshared Nobel prizes is the owner of the idea that high doses of vitamin C are effective against colds and other illnesses. In 1968, he postulated that people's needs much greater than the

Recommended Dietary Allowances (RDAs) for vitamins. He termed this approach "orthomolecular," meaning "right molecule ***(Barrett, 2014).***

Pauling himself reportedly took at least 12,000 mg daily and raised the amount to 40,000 mg if symptoms of a cold appear. In 1993, Pauling said that vitamin C had delayed the cancer's onset for twenty years. In 1976, Pauling and Dr. Ewan Cameron, reported that a majority of one hundred "terminal" cancer patients treated with 10,000 mg of vitamin C daily survived three to four times longer than similar patients who did not receive the same vitamin C supplements ***(Barrett, 2014).***

In August 4, 2008 researchers from the National Institutes of Health (NIH) reported that high-dose injections of vitamin C (4 grams per day) reduce tumor weight and growth rate by about 50 percent in mouse models of brain, ovarian, as well as pancreatic cancers ***(National Institute of Health, 2008).***

Animal studies show that high-dose vitamin C treatment blocks tumor growth in certain models of neoplasm as pancreatic, liver, prostate, and ovarian cancers, sarcoma, and malignant mesothelioma ***(National Cancer Institute, 2015).***

Vitamin C & Stem Cells (SCs):

A) Vitamin C & mesenchymal stem cells (MSCs):

Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity in periodontal ligament stem cells **(PDLSCs)** in a dose dependant manner as low-dose vitamin C treatment (5.0 μg/mL, or 10.0 μg/mL) could not induce hPDLSCs to form cell sheets. However, when treated with 20 μg/mL and 50 μg/mL for 10–13 days in culture, PDLSCs easily became confluent and wrapped at the dish edge; the entire cell sheets were detached smoothly ***(Wei et al., 2012).***

Ascorbic acid can also stimulate MSC proliferation without loss of phenotype and differentiation potency. Ascorbic acid stimulates ECM secretion (collagen and glycosaminoglycan) as well as cell proliferation depending on ascorbic acid concentration ***(Choi & Kyung-Min, 2008).***

B) Vitamin C & embryonic stem cells (ESCs):

Vitamin C can induce differentiation of embryonic stem cells into cardiac myocytes ***(Takahashi et al., 2003).*** As well as, it causes high efficient differentiation of embryonic stem cells (ESCs) into adipocytes ***(Cuaranta-Monroy & Ixchelt, 2014).***

Epigenetically & molecular modification, Vitamin C shows widespread, remarkably specific DNA demethylation of 1,847 genes in human embryonic stem cells (hESCs) ***(Chung et al., 2010).***

At higher levels of gene regulation, MicroRNAs (miRNA) expression is also induced by vitamin C, a non-coding RNAs, involved in post-transcriptional gene regulations that control self-renewal and differentiation of stem cells, which target a class of genes that mainly related to cell differentiation and development. Vitamin C also promotes DNA demethylation and DNA hydroxymethylation especially, at promoter areas leading to activation of pluripotency genes and ESC-specific miRNAs ***(Gao et al., 2015).***

It does those epigenetic modifications through direct regulation of TET enzymes (ten eleven translocation), family of oxidases, activity producing a TET-dependent DNA demethylation and a blastocyst-like state in ES cells **(Figure 1)** ***(Blaschke et al., 2013).***

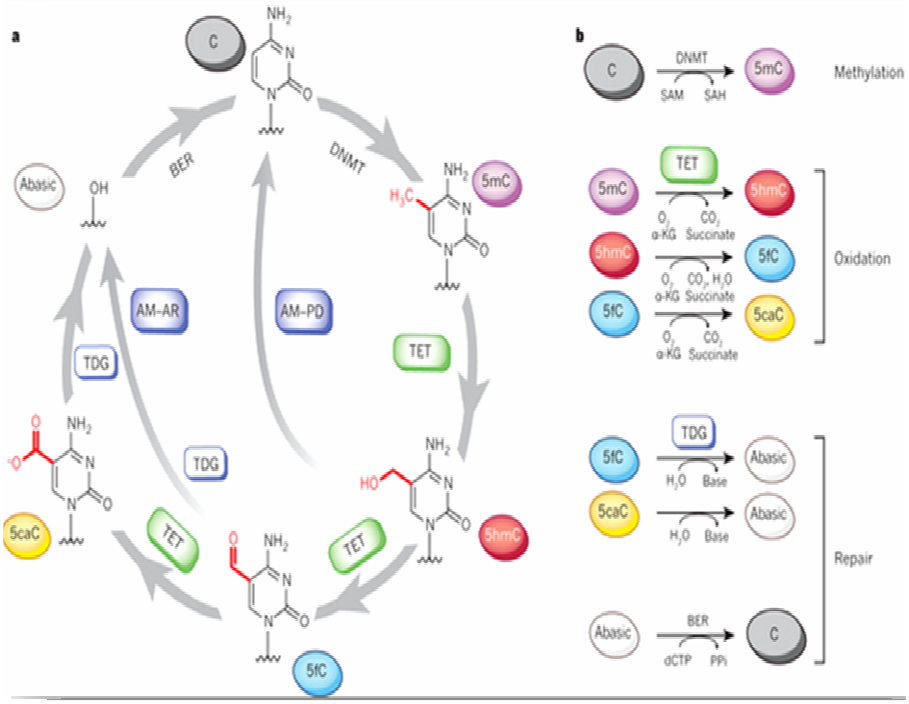

Fig. (1): Showing complete cytosine cycle (methylation, oxidation & repair): **(a)** shows modification of Cytosine within DNA into 5mC bases, introduced by DNA methyltransferase (DNMT) enzymes, then oxidized to 5hmC, 5fC and 5caC. Active modification (AM) followed by passive dilution (PD), 5hmC is diluted in a replication-dependent manner to regenerate unmodified Cytosine. 5fC or 5caC are excised by TDG (thymine DNA glycosylate) generating an abasic site as part of the base excision repair (BER) process that regenerates unmodified C. **(b)**, The individual reactions in the pathway are shown with all reactants. The BER pathway involves excision of the abasic site, replacement of the nucleotide using unmodified deoxycytidine triphosphate (dCTP) by a DNA polymerase (generating pyrophosphate, PPi) and ligation to repair the nick. α-KG, α-ketoglutarate; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine ***(Kohli & Zhang, 2013).***

C) Vitamin C & induced pluripotent stem cells (IPSCs):

Somatic cells could be reprogrammed into induced pluripotent stem cells (iPSCs) by certain factors. Vitamin C can enhance somatic cells reprogramming generating iPSC from both mouse and human somatic cells. As it accelerates gene expression changes and promotes the transition of pre-iPSC colonies to a fully reprogrammed state improving the speed and efficiency of iPSC ***(Esteban et al., 2010).***

It promotes proliferation of cardic progenitor cells enhancing the differentiation of induced pluripotent stem cells into cardiomyocytes with better sarcomeric organization, enhanced responses of action potentials & calcium transients to β-adrenergic and muscarinic stimulations ***(Cao et al., 2012).***

Vitamin C & Liver Regeneration:

In chronic liver disease the hepatocytes regenerative capacity is limited by telomere shortening, resulting in exhaustion of cell regeneration, fibrosis and cirrhosis formation. Telomere shortening & telomerase regulation play an important role on tissue regeneration during aging, chronic diseases & on cancer promotion and progression ***(Carulli & Anzivino, 2014).***

In healthy human liver biopsy samples there is no telomerase activity detected which is closely associated with

expression of its catalytic subunit, telomerase reverse transcriptase (TERT) that increases during liver regeneration, indicating a functional role for telomerase in human liver ***(Sirma et al., 2011).*** Vitamin C has the ability to induce telomerase activity in tissue ***(Wei et al., 2012).***

Because of its anti-oxidant activity, vitamin C intake on acutely intra-lesional liver injury results in reduction of hepatomegaly, lowering the oxidative stress induced by lipid peroxidation ***(Su et al., 2014).***

STEM CELLS (SCS)

Stem cells are a group of cells, with ability to self-renewal and differentiation into various types of cells, for tissue regeneration. There are many types of stem cells, differing in their degree of differentiation and ability to self-renewing **(figure 2)** ***(Ashtiani, 2011).***

Gametes cells (eggs or sperms)**:** stem cells can develop to a whole body after fertilizing. ***Embryonic cells*****:** derived from the part of a human embryo or fetus, are stem cells also with full potential to differentiation. ***Adult stem cells:*** are partially differentiated cells found among specialized (differentiated) cells in a tissue or organ. ***Cancer stem cells:*** are a sub-group of cancer cells that are responsible for escaping of cancer chemotherapy and relapse of tumors. ***Induced pluripotent stem cells (iPS)****:* reveal a special significance, as they can be induced from many adult tissues or organs by modulation of protein factors ***(Ashtiani, 2011).***

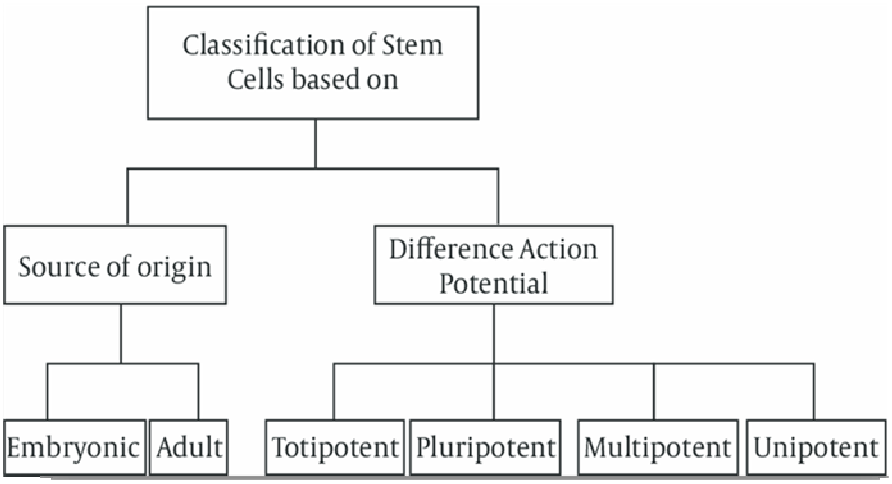

Fig. (2): Classification of stem cells ***(Rostamzadeh et al., 2015).***

Mesenchymal Stem Cells (MSCs):

MSCs are spindle-shaped fibroblast-like cells with ability of self-renewal. In 2006, the International Society for Cellular Therapy proposed minimal criteria to define human MSCs. *First*, MSCs must be plastic-adherent when maintained under standard culture conditions. *Second*, ≥ 95% of the MSC population must express CD105, CD73 and CD90, and lack the expression (≤ 2% positive) of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II surface molecules. *Third*, MSCs must differentiate into osteoblasts, adipocytes and chondroblasts under standard in vitro differentiating conditions ***(Berardis et al., 2015).***

MSCs secrete factors, including IL-6, M-CSF, IL-10, HGF, and PGE2, that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory and immune reactions ***(Li et al., 2013).***

Bone marrow (BM) consists of hematopoietic stem cells and MSCs, BM-MSCs which is found to have high proliferation, and differentiation capacity ***(Truong et al., 2016).***

They have the potential to migrate to the injured site hence to engraft into the concerned organ (*homing*). This involves their ability to migrate across the endothelial cells and to integrate the organ, beside the expression of some integrins, selectins and chemokine receptors involved in the adhesion and migration ***(Salem et al., 2010).***

Mesenchymal stem cells (MSCs) & Liver injury:

Recently, MSCs offer an opportunity to treat both liver inflammatory damage, as well as some chronic liver injury with numerous therapeutic opportunities ***(Nicolas et al., 2016).*** Several studies reported that MSCs has ability to trans-differentiate into hepatocytes ***(Itaba et al., 2015).***

BM-MSCs can markedly decrease the induced liver fibrosis by CCL_4 in albino rats, offering a hope to patient waiting for liver transplantation ***(Ahmed et al., 2014).***

Potential roles of mesenchymal stem cells (MSCs) in liver fibrosis include the following (figure 3):

(1) Trans-differentiation into hepatocyte-like cells.

(2) Suppression of immune reactions.

(3) Secretion of trophic factors to suppress activated HSCs and increases the proliferation of both resident hepatocytes and hepatic progenitor cells.

(4) Anti-fibrosis resulting from the regulation of activated HSCs and immune cells ***(Eom et al., 2015).***

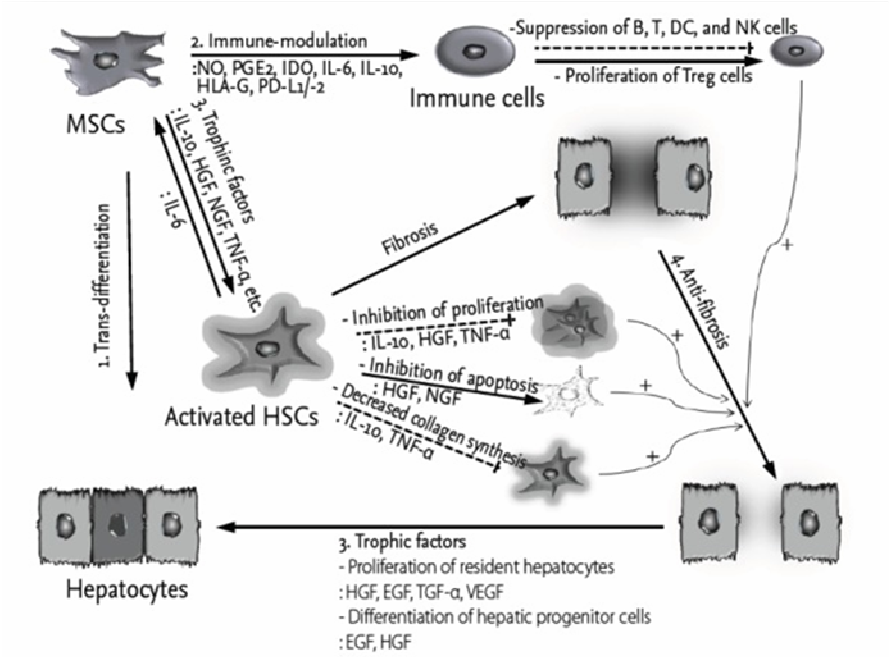

Fig. (3): Roles of (MSCs) in liver fibrosis: Solid lines and dashed lines indicate stimulatory and inhibitory modifications, respectively. The + sign represents stimulatory effects. The shadows represent ECM that is secreted from the HSCs. NO, nitric oxide; PGE2, prostaglandin E2; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; HLA-G, human leukocyte antigen G; PD, programmed death; DC, dendritic cell; NK, nature killer; Treg, regulatory T; HGF, hepatocyte growth factor; NGF, nerve growth factor; TNF-α, tumor necrosis factor α; EGF, epidermal growth factor; TGF-α, transforming growth factor α; VEGF, vascular endothelial-growth-factor ***(Eom et al., 2015).***

LIVER FIBROSIS

The liver is the largest homeoststic organ of the body, with multiple functions, such as detoxification of waste substances, biotransformation of drugs, metabolism of carbohydrates, lipids, proteins, and ethanol. It is also involved in the biochemical processes of growth, providing nutrients and supplying energy. In addition, it is the site of metabolism of carbohydrates and fats, secretion of bile, and storage of vitamins ***(Morales et al., 2015).***

Liver fibrosis is the injury-healing response of the liver to chronic injury. Following repeated injury, the liver undergoes a tissue remodeling and forms fibrosis ***(Zhao et al., 2005).*** With subsequent imbalance of extracellular matrix (ECM) synthesis and degradation mediated by activated hepatic stellate cells (HSCs) ***(Eom et al., 2015).***

Liver fibrosis is a significant health problem with a worldwide mortality attributable to cirrhosis and primary liver cancer of around 1.5 millions death per year. Cirrhosis is the last irreversible stage of fibrosis which occurs mainly in response to viral and toxic-metabolic insults ***(Poynard et al., 2010).***

It is a major public health issue for which no treatment is available that's why cell therapy and, in particular, mesenchymal stem cells (MSCs), represent a promising