**Summary**

The liver is an important organ, responsible for the metabolism, plasma proteins synthesis and detoxification. Liver fibrosis results from the chronic injury to the liver in conjunction with excessive deposition of collagen and other components of extracellular matrix. HSCs play a principal role in liver fibrosis. MSCs transplantation has emerged as a promising therapy for liver fibrosis. HGF has been reported to be a potent agent for acceleration of tissue regeneration following liver fibrosis, but because of its short half-life period, CNP provided the solution to the effective delivery of this factor to its niche.

This work was designed to investigate the possible synergistic effect of HGF incorporated CNP if co-injected with MSCs in improving experimental liver fibrosis and to compare this with the curative effects of both of MSCs and HGF incorporated CNP when they were given separately.

Sixty male albino rats were used in this work and they were divided into two groups:

- **Group I (control group):** Consisted of 10 rats. They were injected intraperitoneally with 1ml sterile distilled water three times weekly for eight weeks. After this time, they were injected with 1ml phosphate buffered saline (PBS) in a single dose into the tail vein.

- **Group II (fibrosis group):** Consisted of 50 rats. They received intraperitoneal injection of 200 mg/Kg of TAA dissolved in 1ml sterile distilled water three times weekly for eight weeks to induce liver fibrosis. After that time, group II (fibrosis group) was further subdivided into five subgroups:

  * **Subgroup IIa (untreated fibrosis group):** Consisted of 10 rats that were injected only with a single dose of 1ml PBS into the tail vein.

  * **Subgroup IIb (MSCs treated group):** Consisted of 10 rats that received a single injection of 1x10⁶ of MSCs Labeled with PKH26 dye in 1ml PBS into the tail vein.

  * **Subgroup IIc (HGF-CNP treated group):** Consisted of 10 rats that received a single injection of HGF-CNP in a dose of 100 nanogram HGF carried on 1ml CNP solution into the tail vein.

  * **Subgroup IIId (MSCs + HGF-CNP treated group):** Consisted of 10 rats that received a single injection of 1x10⁶ PKH26 Labeled MSCs in 1ml PBS mixed with HGF-CNP in a dose of 100 nanogram HGF carried on 1ml CNP solution into the tail vein.

  * **Subgroup IIe (CNP treated group):** Consisted of 10 rats that received a single injection of 1ml CNP solution into the tail vein.

Four weeks after all injections, all the sixty rats were sacrificed and liver specimens were taken rapidly and processed to prepare paraffin sections. Sections were stained with
H&E, Masson’s trichrome, α–SMA immunostain and PCNA immunostain. Fluorescent microscope was used to detect PKH26 labeled MSCs.

Morphometric assessment of the area% of collagen and α–SMA immunopositive cells as well as the number of PCNA immunopositive cells were performed using image analysis system. Statistical analysis of morphometric measurements, comparison between groups using ANOVA test and comparing each two groups using post Hoc test was done.

The present study, group I (control group) showed the normal hepatic architecture in which cords of normal hepatocytes radiating from the central vein, forming ill-defined hepatic lobules. Normal portal areas situated at the peripheries of these lobules. Minimal amount of collagen could be detected only around the central vein and in the portal areas. α–SMA immunoreaction was found only in the media of the central veins and vessels of portal areas. Scarce proliferating hepatocytes were detected with PCNA immunostaining.

In subgroup IIa (untreated fibrosis group), the effect of TAA appeared obviously in the form of marked disorganization of the liver tissue accompanied with many apoptotic and vacuolated hepatocytes. Heavy inflammatory cellular infiltrate was also found. Compared to control group, significant increase in the collagen fibers in the interlobular septa, portal areas and in between the hepatocytes was noticed in untreated fibrosis group. α–SMA positive HSCs showed significant increase in area% in between the hepatocytes. Few proliferating hepatocytes were detected by PCNA immunostaining.

Subgroup IIb (MSCs treated group) showed partial recovery from the fibrotic state of the liver in the form of increased amount of normal hepatocytes compared with the untreated fibrosis group but there was mild inflammatory cellular infiltrates. Collagen fibers in the interlobular septa and portal areas exhibited significant decrease in amount. Significant decrease in α–SMA positive HSCs in between hepatocytes and in portal areas was detected (as compared to untreated fibrosis). PCNA immunostaining revealed significant increase in proliferating hepatocytes number.

Subgroup IIc (HGF-CNP treated group) showed moderate improvement of liver fibrosis by reappearance of normal hepatocytes but few vacuolated and apoptotic cells and mild inflammatory cellular infiltrates were found. Moderate to large amount of collagen was seen within the connective tissue septa and in the portal areas; however it showed significant decrease in amount when compared with the untreated fibrosis group. α-SMA positive HSCs showed significant decrease in area% while PCNA immunoreaction exhibited significant increase in number (all compared to untreated fibrosis group).

Subgroup IId (MSCs+HGF-CNP treated group) revealed marked improvement of liver fibrosis with regaining of many normal hepatocytes but with the exception of few remained apoptotic cells. Significant decrease in collagen amount was observed, similarly significant decrease in HSCs immunoreactive to α–SMA(as compared to untreated fibrosis group). PCNA immunostaining revealed significant increase in proliferating hepatocytes number (as compare to untreated fibrosis group).
Subgroup Ile (CNP treated group) showed minimal effect of CNP in improving liver fibrosis. The liver sections revealed loss of the lobular architecture with the presence of many vacuolated and apoptotic cells. Large amount of collagen fibers were found in the interlobular septa, portal tracts and in between the distorted hepatocyte cords. Extensive α–SMA positive HSCs were present in between the hepatocytes while few proliferating hepatocytes were detected by PCNA immunostaining.

PKH-26 labeled MSCs were detected only in MSCs treated and MSCs+HGF-CNP treated groups.