Transplantation of Modified and Fresh Hepatocyte Reduces Hepatotoxicity Induced by Carbon Tetrachloride

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Abstract: Hepatocyte transplantation (HCT_X) is a strategy that has potential as a supportive treatment regimen and in some cases, an alternative to orthotopic liver transplantation (OLT) for a variety of liver disorders. Carbon tetrachloride (CCl_4) intoxification in rodents is a commonly used model of both acute and chronic liver injury; it causes hepatocyte injury that is characterized by centrilobular necrosis and steatosis. This study demonstrates the therapeutic effect of fresh and microencapsulated hepatocytes in reduction of hepatotoxicity induced by CCl_4 in hamsters administered a dose of 0.02 ml of 10% of CCl_4 dissolved in corn oil /100 gm. body weight, for 24 hrs. Four groups each has ten hamsters ***(GI)***- normal control uninjected, ***(GII)***-negative control injected with CCl_4 toxic dose intraperitoneally (i.p) for 24 hrs., ***(GIII)***-injected with CCl_4 toxic dose (i.p) for 24 hours, treated with cyclosporin as immuno-suppressive drug 24 hrs. post injection, then transplanted with fresh hepatocytes intrasplenically and ***(G IV)***-injected with CCl_4 toxic dose (i.p) for 24 hours, then transplanted with modified hepatocytes encapsulated in sodium alginate intrasplenically. Freshly isolated hepatocytes with a mean viability 92.97±1.2% were used for microencapsulation and transplantation. The mortality rate after hepatocytes transplantation in hamster groups (III and IV) was 50% but in negative control group was 90%. The biochemical parameters shows correction in liver function after transplantation of either fresh or microencapsulated hepatocytes comparing to negative control groups beside correction in oxidative stress and lipid peroxidation. Histological study showed the presence of transplanted hepatocytes in spleen of recipient. The conclusion of the above study showed that the animal groups that transplanted with microencapsulated hepatocytes survived without any immune rejection for the same period in comparison with fresh hepatocyte transplantation treated with immunosuppressive drug (cyclosporin), beside semi similar liver function recovery.

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1. Introduction

Human is exposed every day to certain toxic chemicals and pathogens, which can cause certain serious health problems. Liver plays central role in transformation and clearance of these agents. Metabolism in the liver protects tissues in higher organisms from potentially harmful environmental chemicals; the metabolic products of detoxification reactions that protect other tissues from effects of the primary toxicant can be destructive to the liver when in excess. Certain medicinal agents when taken in over doses and sometimes even when introduced within therapeutic ranges may injure the organ and other chemicals such as agents used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins (Achiliya *et al*., 2003) amongst them is carbon tetrachloride. Carbon tetrachloride is a colorless liquid, non-flammable, and is heavier than air (The World Book Encyclopedia, 1992); it was once widely used as a cleansing fluid in households and as solvent for oils, amongst other uses. The most important use of carbon tetrachloride is as an intermediate in the preparation of Freon (CCl_2F_2), which is used as a refrigerant and as a propellant in aerosol bombs (Price, 1980). Carbon tetrachloride is very toxic and because of this, most of its uses in households and industries have been suspended. The main route of exposure of humans and animals to it includes inhalation, ingestion, and absorption. Once entered the body, it causes a lot of injury to the organs of the body (Reynolds *et al*., 1984) including the lungs, heart, gastrointestinal tract, kidneys, central nervous system, and liver (ATSDR, 2003). Of concern here is the adverse effect of carbon tetrachloride to the liver treating prominent functions for survival; thus ingestion of carbon tetrachloride can lead to marked hepatotoxicity (Liu *et al*., 1995).

Carbon tetrachloride (CCl_4) intoxication in rodents is a commonly used model of both acute and

chronic liver injury (Chundong *et al.*, 2002). Administration of carbon tetrachloride (CCl_4) to rodents is a widely used model to study mechanisms of hepatic injury; it causes hepatocyte injury that is characterized by centrilobular necrosis and steatosis (Recknagel, 1987) that is followed by hepatic fibrosis and cirrhosis. Hepatic injury is induced in two phases, the initial phase is generation of radicals and the second phase is activation of Kupffer cells (Edwards, 1993) which release various proinflammatory mediators (Ramadori *et al.*, 2008).

Different concentrations of CCl_4 could be used to cause various degrees of liver damage, thereby generating an ideal hepatotoxicity model organism. Shehu (2008) used a certain concentration of CCl_4 to induce liver damage followed by natural healing which started at three days of the liver damage inducement. It had an effect on biochemical characteristics such as increased lipid peroxidation and the activities of aspartate transaminase (AST) and alanine transaminase (ALT) (Recknagel, 1987).

The treatment of acute and chronic liver failure is still a challenge despite modern innovations in therapy. Liver transplantation can restore liver function and improve patient survival but donor shortages limit this treatment to a small number of patients. Several approaches are being developed to overcome this limitation. These include bio artificial livers (Abd El-Latif *et al*, 1995), cellular transplantation (of human hepatocytes or progenitor cells), and whole liver xenotransplantation. Another option currently under development is the transplantation of cells from another species, termed xenotransplantation (Mohamed *et al.*,2001). Cellular transplantation has emerged as an alternative for treating liver failure especially in patients in critical condition. Fisher (2006) and Sgroi (2009) addressed the clinical studies that have used human adult hepatocytes to treat acute and chronic liver diseases and demonstrated improvements in neurological status and biochemical parameters, with some patients presenting full recovery and others being successfully bridged to liver transplantation (Sgroi, 2009). Cell transplantation has been used for temporary metabolic support of patients in end-stage liver failure awaiting whole organ transplantation, as a method to support liver function and facilitate regeneration of the native liver in cases of fulminant hepatic failure; and in a manner similar to gene therapy, as a (cellular therapy) for patients with genetic defects in vital liver functions, hepatocytes could be readily available in sufficient quantities to treat patients in critical condition and thereby compensate for the donor shortage. Therefore, hepatocyte transplantation may be a promising approach treating liver disorders.

Liver has been considered as an optimal site for hepatocyte transplantation; however even in this organ, the survival rate of transplanted hepatocytes is extremely low in comparison to spleen as hepatocytes are well engrafted when injected into splenic pulp and they are entrapped in the sinusoid and vascular spaces (Akhter, 2007). The spleen is considered as the most privileged anatomical site for hepatocyte transplantation. It is accessible by means of laparotomy. It can entrap a limited, but a sufficient number of hepatocytes within its sinusoids, providing conditions very similar to natural cell microenvironment. This process has been described by Mito *et al.*,(1979), by the term “splenic hepatization”. The main obstacle for wider usage of hepatocytes transplantation is the immune rejection or their rapid elimination by recipient macrophage (Gewartowska, 2007). The use of microencapsulated cells can reduce the immune rejection response (Bonavita *et al.*, 2010), microencapsulating technique initiated by Lim and Sun (1980) brought new hopes for artificial liver and hepatocyte transplantation as a result of immunoisolation of these cells as retaining in a semi permeable membrane both protects them from immune system and maintain their survival and metabolic function (Akhter, 2007).

In this study, a hamster model of hepatotoxicity was induced by injection with a toxic dose of carbon tetrachloride. The therapeutic efficacy of hepatocyte transplantation (HCTx) either fresh or microencapsulated in splenic pulp is investigated through biochemical and histological examinations.

2. Material and Methods

Animals

Seventy male Syrian golden hamsters, average weight 110gm ± 20, were bred and maintained at the Schistosome Biological Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Animals were housed in a controlled temperature and light environment, and were given water and commercial chow *ad libitum*. The animal experiments were conducted at the animal unit according to the international ethical guidelines for the care and use of animals for research purposes.

Carbon tetrachloride induced hepatotoxicity

Preliminary experiment: The most effective dose of 10% CCl_4 (prepared by dissolving 10 ml of CCl_4 dissolved in 90 ml of corn oil) **(**Kumata et al.,1975) which causes hepatotoxicity with lower mortality rate was tested. Three groups of animals ten hamsters each, were intraperitoneally injected with CCl_4 in different doses as follows: ***(G1*** - 0.02ml/100 gm. body weight, ***(G2)***- 0.3ml/100 gm. body weight and ***(G3)***-0.7ml/100 gm. body weight.

Experimental design

Forty hamsters, as model of liver cirrhosis using CCl_4 toxic dose (0.02 ml/100gm.body weight), were

classified into 4 sub-groups; each group has ten hamsters as follows***: (GI)***- normal control uninjected, ***(GII)*** – negative control injected with CCl_4 toxic dose intraperitoneally (i.p) for 24 hrs., ***(GIII)***-Injected with CCl_4 toxic dose (i.p) for 24 hours, treated with cyclosporin as immuno-suppressive drug 24 hrs.post injection, then transplanted with fresh hepatocytes intrasplenically and ***(GIV)***-Injected with CCl_4 toxic dose (i.p) for 24 hours, then transplanted with modified hepatocytes encapsulated in sodium alginate intrasplenically.

Hepatocytes isolation

Fresh hepatocytes were isolated using Seglen's *in situ* collagenase perfusion technique (Seglen, 1979), in which perfusion of the liver was done firstly with Krebs Ringer Buffer "KRB" (137 mmol. NaCl, 5.3 mmol. KCl, 0.8 mmol. Mg $SO_4.7H_2O$, 0.4 mmol. Na_2HPO_4, 0.4 mmol. KH_2PO_4, 5.5 mmol.Glucose, and 5 mmol. HEPES), then liver was digested by collagenase buffer (100 ml of KRB, 0.025 gm. $CaCl_2$, and 0.05 gm. collagenase). After isolation of hepatocytes, count and test of viability of yield cells was performed by using 0.4% trypan blue exclusion dye.

Microencapsulation of hepatocytes

Isolated cells were microencapsulated according to the Lim and Sun (1980) and Fritschy (1991) methods with modifications. The hepatocytes were centrifuged at 1700*g* for 2 minutes before being resuspended in 4% sodium alginate (ALG) in normal saline solution (pH 7.2) at a concentration of 1 x 10^7/ml. The suspension was stirred adequately into a homogeneous mixture before being injected into the syringe pump and dropped into 100 mmol/L calcium chloride solution (HEPES buffered, pH 7.2). Droplets were then washed in 4°C saline and reacted in sequence with 0.05% Poly-L-Lysine (PLL) for 8 minutes, Droplets were then rewashed in 4°C saline, and reacted with 0.2% (w/v) ALG for 4 minutes, then rewashed again in saline and finally reacted with 30 mmol/l sodium citrate (SC) for 8 min.

Transplantation of fresh and microencapsulated hepatocytes

Fresh and microencapsulated hepatocytes were suspended in sterile normal saline in aseptic conditions. Transplantation of one million-cell suspension of fresh and microencapsulated hepatocytes was done by injection of cell suspension slowly into hamster's spleen. For intrasplenic transplantation of hepatocytes, hamsters were anesthetized and a small surgical incision was made in the flank and the spleen was exposed. Freshly harvested hepatocytes suspended in normal saline were injected into the inferior pole of the spleen using a 25-gauge needle connected to a 1ml syringe without constriction of the blood vessels. Homeostasis was secured with a ligature around the spleen proximal to the injection site. Before transplantation of fresh hepatocytes by 24 hrs. hamsters were treated with cyclosporine as immunosuppressive drug.

Assessment of histopathology

Murine livers and spleens recovered from hamsters were fixed in 10% buffered formalin and processed to paraffin blocks. Liver sections (4 µm thick) were cut 250 µm apart from the proceeding sections. Five paraffin liver sections were prepared from each animal and stained with hematoxylin and eosin (H&E) and histology was studied.

Estimation of biochemical parameters

Liver marker enzymes such as alanine aminotransferase (ALT),aspartate aminotransferase (AST) (Ramadori *et al.*,2008) total proteins (TP) (Vasndhe *et al.*,2006) total bilirubin (TB) (Gornall *et al.,1949)* and alkaline phosphatase (ALP) were estimated in the collected sera (Belfield and Goldberg 1971).

Assessment of oxidative stress and lipid peroxidation

Concentrations of reduced glutathione (GSH) (Ellman, 1959), superoxide dismutase (SOD) (Walter and Gerade 1970) and lipid peroxidase (MDA) (Ohkawa, 1979) were estimated in the collected sera.

Statistical analysis

Results were analyzed with the use of SPSS software (Version 16.0). Values were expressed as mean±standard error of the mean. Means of groups were compared with the use of an unpaired *t*-test. For comparison of more than 2 groups, an ANOVA test was used. Data were considered significant at *P* value <0.05.

3. Results

Hepatotoxicity induction by CCl_4

Detection of the effective dose which induced hepatotoxicity with low mortality rate is presented in (Fig.1). The mortality rate of three different doses of 10% CCl_4 (0.7, 0.3 and 0.02 ml/100 gm. body weight) 5 days post injection is 8/10 (80%), 6/10 (60%) and 3/10 (30%) respectively. The optimal dose used therefore was 0.02 ml /100gm. body weight.

Number and viability of isolated hepatocytes

A mean of approximately 134±38.8 ×10^6 of liver cells was harvested from each hamster donor, with a mean viability about 92.97±1.2%. Hepatocytes isolation from normal liver and their microencapsulation is shown in (Fig. 2A and 2B).

Mortality rate

The mortality rate of different groups of hamsters after injection with 0.02 ml/100 gm. body weight intraperitoneally is presented in (Fig.3).Negative control group had 9/10 (90%) mortality 10 days post injection, but animal groups transplanted with freshly

isolated and microencapsulated hepatocytes had 5/10 (50%) mortality 10 days post transplantation.

Biochemical study

Table (1) presents changes of the biochemical parameters before and after hepatocyte transplantation either fresh or microencapsulated. Hamsters injected with toxic dose of CCl_4 produced significant elevation (P<0.05) in the serum concentrations of ALT, AST, ALP and total bilirubin in (Figs 4,5,6 and 7), and a marked depletion in the hepatic content of total proteins and GSH in (Figs.8 and 9), with significant elevation in SOD activity and MDA level compared to their corresponding normal levels presented in (Figs.10 and 11). After transplantation of hepatocytes either fresh or microencapsulated, these serum and hepatic markers related to liver damage were approximately restored to their normal levels in most test parameters. Moreover, the serum biochemical indeces insignificant between the fresh and the microencapsulated groups in most test parameters.

Table 1: Effect of usage of fresh and microencapsulated hepatocytes (HCTx) on serum liver enzymes, antioxidants activity and lipid peroxidation of liver hepatotoxicity induced by Carbon tetrachloride.

Animal Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/l)	TP (g/dl)	TB (mg/dl)	GSH (mmol/l)	SOD (u/ml)	MDA (nmol/ml)
Normal (N=5)	18.3±0.9$^{¥}$	15.04±1.06$^{¥}$	27.9 ± 1.2$^{¥}$	6.9 ±0.58$^{¥}$	0.78±0.01$^{¥}$	1.68±0.09$^{¥}$	53.84±2.23$^{¥}$	8±0.73$^{¥}$
Negative control (N=10)	112.4±6.8*	88.6±6.8*	137.4±7.7*	3.21±0.16*	2.12±0.19*	0.27±0.01*	203.7±5.37*	26.48±1.7*
Fresh HTC_X (N=10)	26.8±1.6$^{¥}$	22.22±3.2$^{¥}$	62.16±3.4$^{¥*}$	4.7±0.24$^{*¥}$	1.16±0.2$^{¥}$	0.78±.059$^{¥*}$	142.9±2.41$^{¥*}$	12.87±0.42$^{¥*}$
Microencapsulated HTC_X (N=10)	22.6±7.4$^{¥}$	30.64±3.7$^{*¥}$	52.2±4.44$^{*¥}$	4.96±0.6$^{*¥}$	1.36±0.06$^{¥}$	0.852±0.08$^{¥*}$	134.8±9.3$^{¥*}$	12.1±1.87$^{¥*}$

Values are presented as mean ± SE. Hamsters injected with CCl_4 (0.02ml/100 gm. body weight) 48 hrs. before hepatocyte transplantation took place.

Significantly difference from the corresponding at P<0.05*normal, and ¥ negative control at P<0.05.

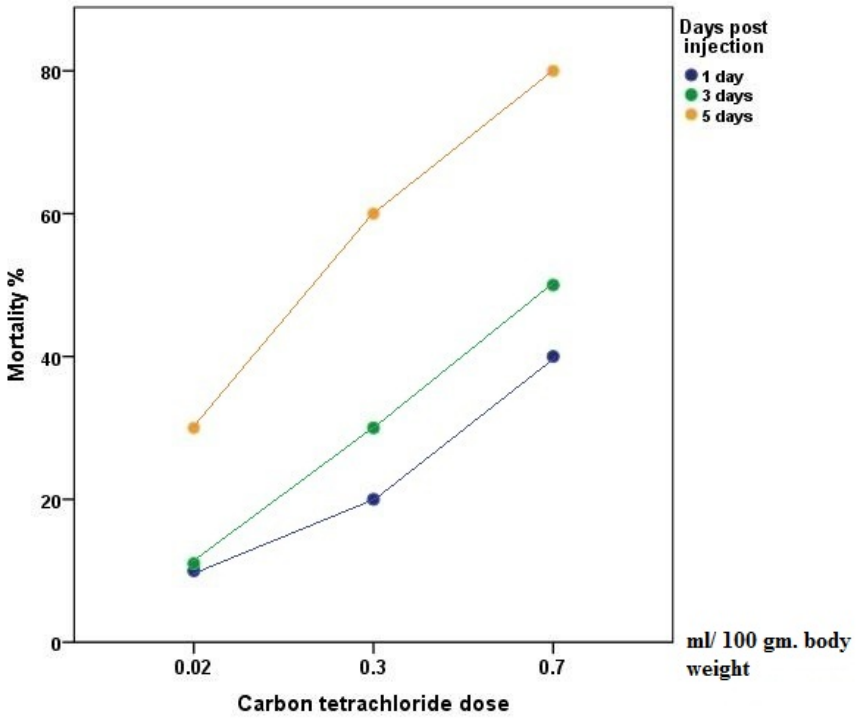

Fig 1: Effective dose of carbon tetrachloride which induced hepatotoxicity with low mortality rate

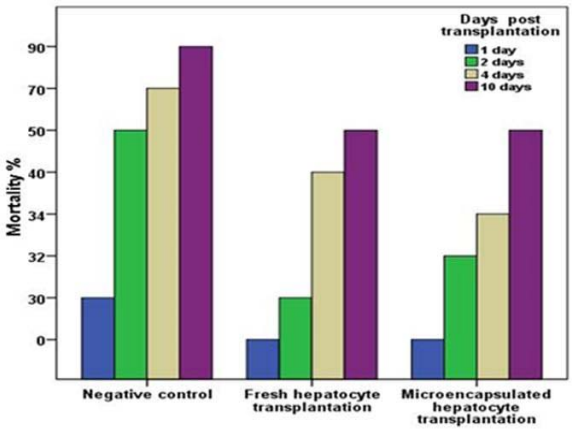

Fig 3- Mortality Rate after Transplantation.

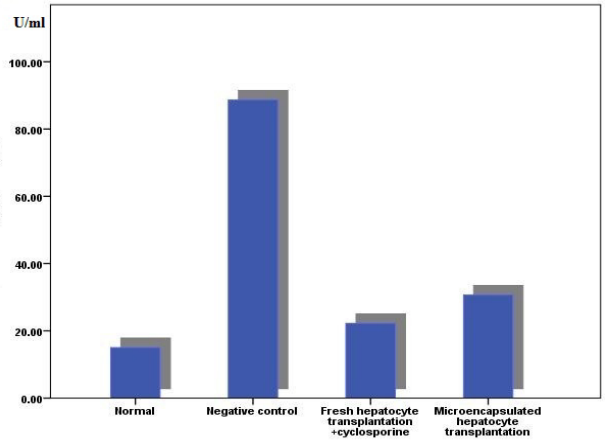

Fig 4: ALT concentration estimated in different groups of CCl_4 hepatotoxicity

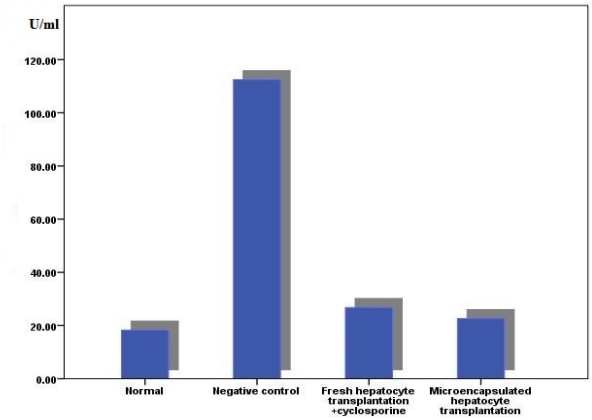

Fig 5: AST concentration estimated in different groups of CCl_4 hepatotoxicity

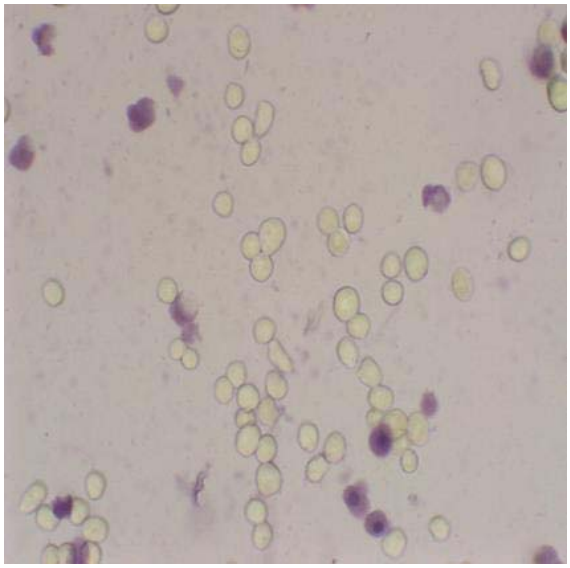

Fig 2-A High power magnification of hepatocytes showing numerous viable cells excluding trypan blue stain and few darkly stained (dead) cells which failed to exclude the dye (X40).

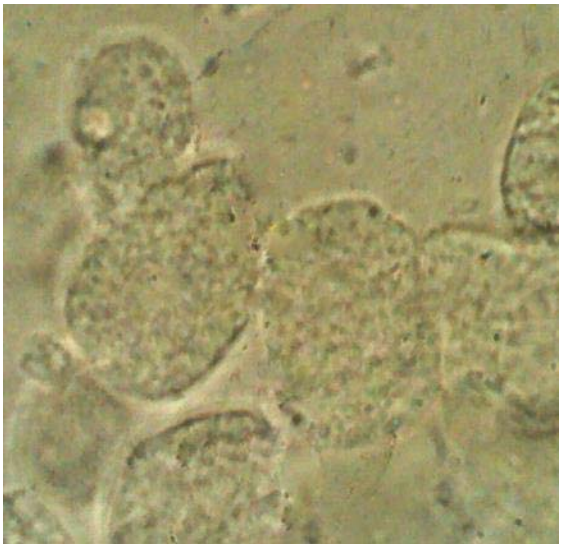

Fig 2-B High power magnification of alginate capsule filled hepatocytes, the solution is almost clear of deformed or extremely small beads (X100 oil lens)

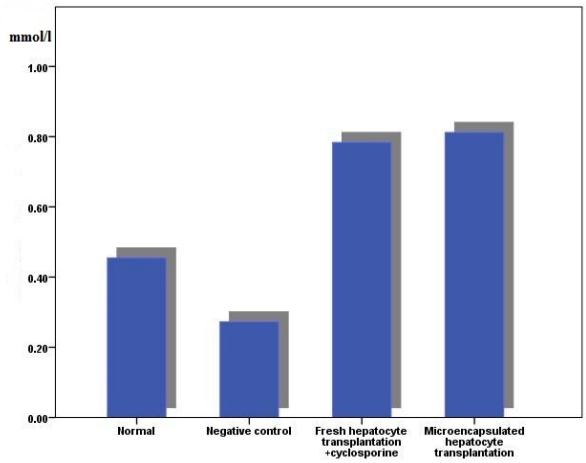

Fig 9- GSH concentration estimated in different groups of CCl_4 hepatotoxicity

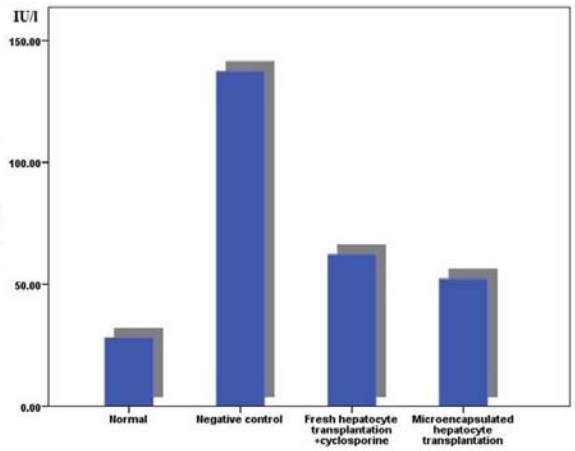

Fig 6- ALP concentration estimated in different groups of CCl_4 hepatotoxicity

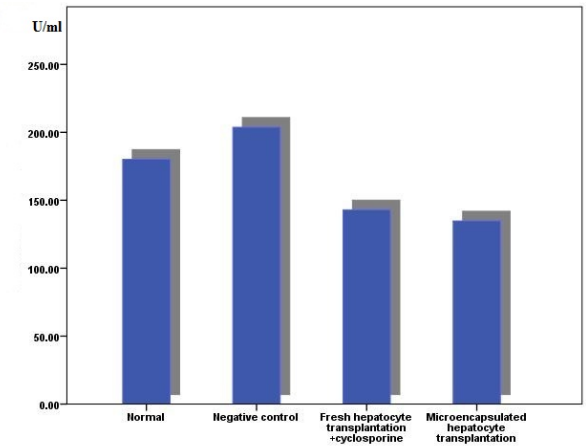

Fig 10-SOD concentration estimated in different groups of CCl_4 hepatotoxicity

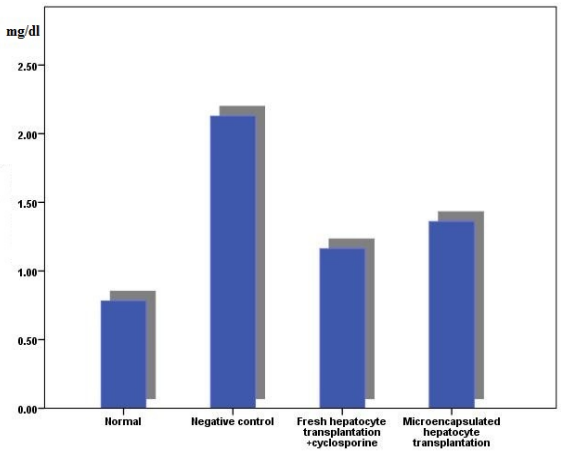

Fig 7-: TB concentration estimated in different groups of CCl_4 hepatotoxicity

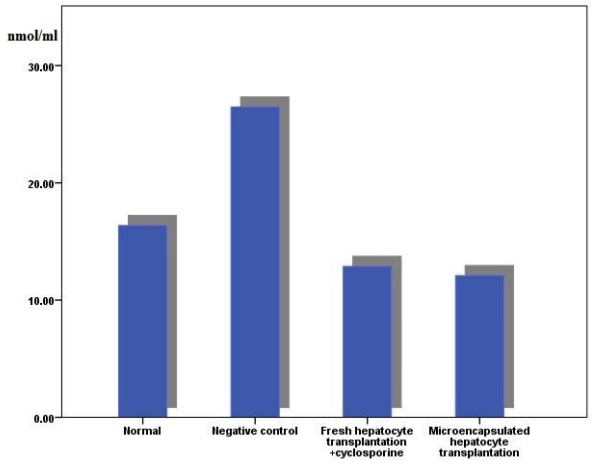

Fig 11: MDA concentration estimated in different groups of CCl_4 hepatotoxicity

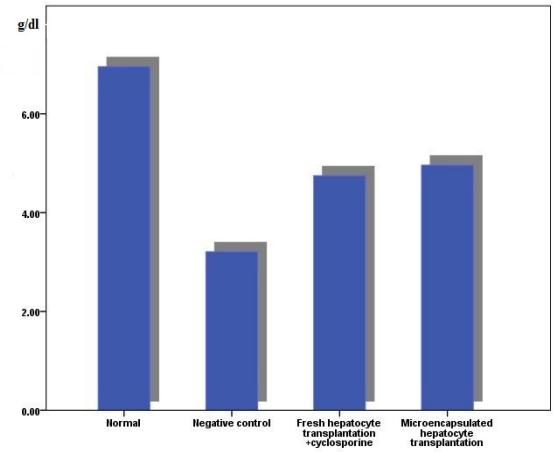

Fig 8- TP concentration estimated in different groups of CCl_4 hepatotoxicity

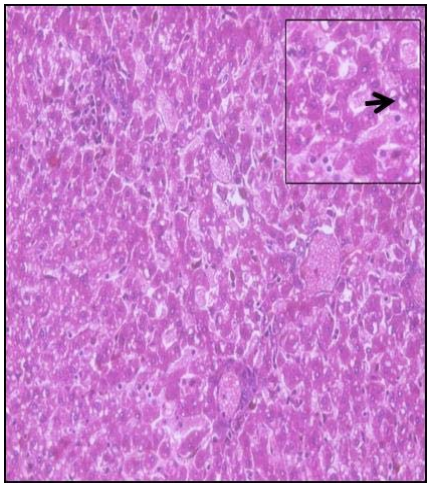

Fig12 B: - Injected with CCl_4 (0.02ml/100 gm. body weight) hamster's liver section scarified 48 hrs. post injection, showed moderate hydropic degeneration of hepatocytes and micro steatotic changes (arrow) and mild congestion of sinusoids (H&E,x100).

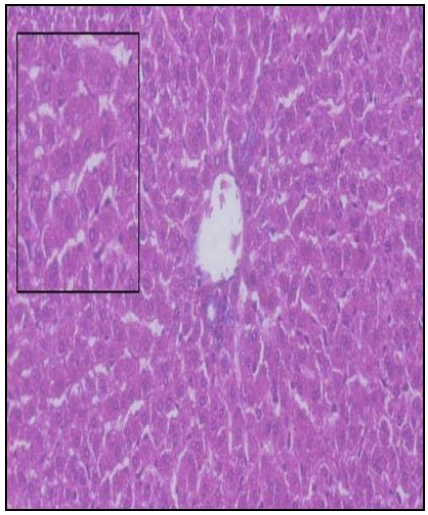

Fig12 A: Normal uninjected liver section, showed sinusoid between hepatic plates (H&E, x100).

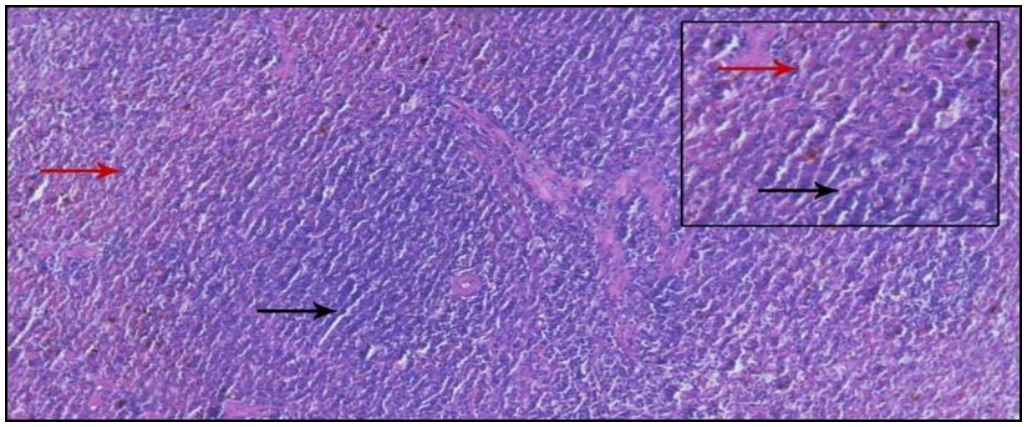

Fig 13 A- Spleen section: Normal uninjected showed red pulp (red arrow), and white pulp (black arrow) (H&E, x100).

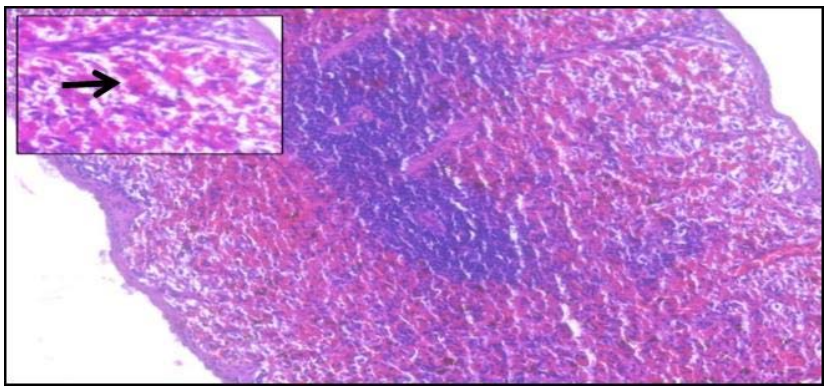

Fig 13 B- Spleen section: Injected with CCl_4 (0.02ml/ 100 gm. body weight) and transplanted with fresh hepatocytes showed red and white pulp of the spleen entangling in between scattered rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (arrows) (H&E, x100).

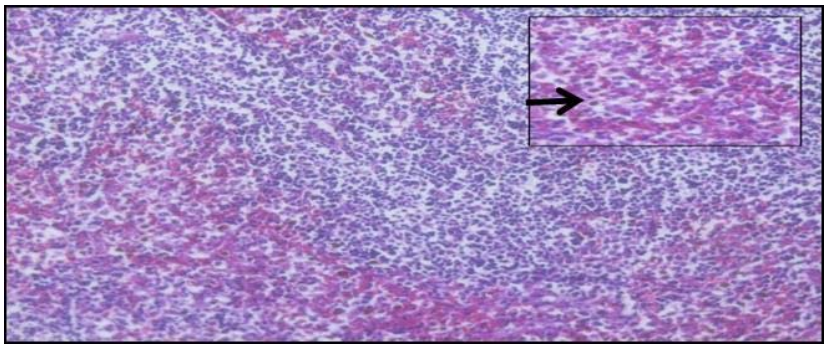

Fig 13 C- Spleen section: Injected with CCl_4 (0.02ml/ 100 gm. body weight) and transplanted with microencapsulated hepatocytes showed red and white pulp of the spleen entangling in between scattered rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (arrows) (H&E, x100).

Histological study

Liver histopathology: Normal liver shows arrangement of hepatocytes in lobules and sinusoids between hepatic plates called sinusoid (Fig.12A). Hepatic tissues of negative control hamster group showed moderate hydropic degeneration of hepatocytes, micro steatotic changes and mild congestion of sinusoids (Fig.12B).

Spleen histopathology: Normal spleen showed red and white pulp (Fig.13A). After intrasplenic injection with either fresh or microencapsulated hepatocytes, and sacrified 10 days post transplantation, it showed rounded or hexagonal epithelial cells with rounded nucleus and esinophilic cytoplasm (hepatocytes like cells) (Fig.13B and C).

4. Discussion

Orthotopic liver transplantation (OLT) is the only therapy proven to directly alter mortality, and therefore, remains the ultimate standard of care for liver disease patients (Brown et al., 2005). The expanding use of adult living donors may abate the organ shortage to some extent, but this procedure is not without significant risk to the donor and recipient (Goldstein et al., 2003). Furthermore, patients who do receive transplants are subjected to the costs and complications associated with major surgery as well as a life-time immunosuppressive regimens. These complications can range from simple infections to renal failure, hyperlipidemia, and an increased incidence of skin and other types of cancers following long term immunosuppression. As with all other organs, the number of liver donors does not nearly equal the number of patients on the waiting list (Stephen et al., 2008) .Consequently; alternative approaches are actively being pursued. These include non-biological extracorporeal systems, such as hemoperfusion, hemodialysis, plasma exchange, and plasma pheresis over charcoal or resins. For providing the large array of known and currently unidentified liver functions, cell-based therapies have been proposed as an alternative to both liver transplantation and strictly non-biological systems. These cell-based therapies range from approaches that provide temporary support, such as Bio Artificial Liver (BAL) devices, to more permanent interventions, such as cell transplantation as hepatocyte transplantation (Allen,2002) .

Transplantation of isolated liver cells (hepatocytes) is a less invasive alternative to whole organ transplantation or as a “bridge” while awaiting the availability of a donor liver. It presents many advantages over OLT. For instance, cells from a single liver donor can be used by many recipients (Grompe et al.,1999). Freshly isolated hepatocytes have been shown to perform better than cryopreserved cells in many studies (David et al., 2001 and Jamal et al.,2000).

Hepatocyte transplantation therapy is less invasive than organ transplantation. It could circumvent immunosuppressive regimens through the use of autologous cells (Gupta,2002).

Several reports have demonstrated the feasibility and efficacy of cell transplantation in providing specific function in various experimental animal models of human disease. However, without adequate immunosuppression, complications due to tissue rejection remain a significant problem. Microencapsulation of cells within a synthetic semipermeable membrane, prior to transplantation, has been proposed for circumventing immunological complications following transplantation. The microcapsule's semipermeable membrane allows permeant molecules to freely diffuse across while preventing the microencapsulated cells from escaping. This membrane also keeps unwanted substances, such as cells and antibodies, from entering the microcapsule. Thus, microencapsulation provides an innovative and

unique technique for the transplantation of foreign tissue and cells without the need for immunosuppression drugs (Dixit and Gitnick 1995).

In the current study, 1 x 10^6 isolated hepatocytes with viability mean 92.97±1.2% were used for transplantation. This is consistent with that of Badrawy (2002) who found successful hepatocyte engraftment in rats when cell viability was higher than 85%[l]. Our study examined the hepatocytes derived from donor liver and were capsulated by the alginate polylysine alginate (APA) microencapsulation technique. The obtained microcapsules exhibited a good smooth surface and integrated appearance. Furthermore, living cells inside the microcapsules were > 90% as determined by trypan blue staining.

The mortality rate after injection with toxic dose of CCl_4 in hamsters was 90% 10 days post CCl_4 administration but hamsters which were transplanted with fresh or microencapsulated hepatocytes after CCl_4 toxicity were nearly the same as 50% for each respectively. Although the survival rate between the two transplanted groups was similar to each other, the transplantation of microencapsulated hepatocytes was more beneficial to avoid the use of immunosuppressive agents with their known undesirable effects (Hernandez *et al*., 2010). A better survival rate of intrasplenically transplanted hamsters has been demonstrated, suggesting the superiority of the spleen as a site for cell implantation in this experimental model. Pilichos (2004) stated that, the rats intrasplenically transplanted with hepatocytes succeeded to survive until day 6 post transplantation (Pilichos *et al*., 2004).

Carbon tetrachloride CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical (Johnson and Kroening 1998). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 (Kaplowitz *et al.,* 1986). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin and decrease in total proteins. The diagnosis of organ disease/damage is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of cellular damage, the intracellular concentration of the enzymes and the mass of affected tissue and the concentration of the enzyme released reflect the severity of the damage. ALT and AST are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die transaminases are released into blood stream, where they can be measured. They are therefore the index of liver injury (The World Book Encyclopedia,1992). In this study, treatment of hamsters with toxic dose of CCl_4 resulted in a significant increase in both ALT and AST than normal un-injected. This result is in accordance with Achliya (2003) and Harish (2006) who stated that carbon tetrachloride administration caused damage in cell membrane, change enzymes activity, induce hepatic injury of necrosis and finally results in cell death. They suggested that the hepatic injury caused flow of soluble proteins of the cells into serum including transaminases. After transplantation of hepatocytes either fresh or encapsulated there were significantly better outcomes in serum transaminases; these levels were still reduced compared to negative control groups. These findings are in accordance with Bin (2012) who stated that two weeks after hepatocytes transplantation into the rats, serum levels of transaminases were significantly reduced.

Serum ALP, TB and TP levels on the other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel and Garcipiana 1992). In current study, hamsters who received a toxic dose of CCl_4 developed significant hepatic damage as evidenced by substantial increases in the serum activities of ALP and TB than normal in agreement with Mukherjee (2003). Also after injection with toxic dose of CCL_4 we found significant decrease in serum level of TP in accordance with Rao (2006). After transplantation of hepatocyte there were significantly better outcomes in serum ALP, TB and TP levels.

The study of lipid peroxidation is attracting much attention in recent years due to its role in disease processes (Kale and Sitasawad, 1990). The early event following liver injury is the production of excessive reactive oxygen species (ROS) which induced hepatic oxidative stress. It was associated with over production of MDA one of the final products of lipid peroxides in host cells (Mohamed et al., 2008). The results detected higher levels of MDA in the serum of CCl_4 injected hamsters than in normal ones which confirms that CCl_4 induces lipid peroxidation and liver damage in agreement with Robbins and Cotran (2006). After transplantation of either fresh or encapsulated hepatocytes there was significant decrease in serum MDA.

The antioxidant enzyme superoxide dismutase (SOD) keeps homeostasis and protects against oxidative stress damage by removing toxic free radicals in vivo. The results obtained showed elevation activity of SOD after liver injury in accordance with Jia (2009). SOD and MDA levels were increased after hepatocyte

transplantation than normal; these data were in agreement with Hassan, 2005 who stated that reactive oxygen species (ROS) play a central role in ischemia reperfusion injury after organ transplantation which appears as increased SOD activities after liver transplantation, and correlated with MDA.

Reduced glutathione (GSH) is a major nonprotein thiol in living organism, which plays a central role of coordinating the body's antioxidant defense process. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds such as free radicals. Glutathione status is a highly sensitive indicator of cell functionality and viability. Perturbation of GSH status of biological system has been reported to lead to serious consequences (Pastore,2003). After injection of hamsters with a toxic dose CCl_4, GSH concentration was lower than normal; these data are in agreement with Alisi[5]. After transplantation of either fresh or encapsulated hepatocytes there was significant decrease in serum GSH (Fisher and Strom 2006).

Conclusions

Transplantation of hepatocytes in hamster has shown reconstitution of defective hepatic enzymes, improved survival rate and improved oxidative stress and lipid peroxidation in hepatotoxicity model induced by carbon tetrachloride. Microencapsulation of hepatocytes in sodium alginate capsule can overcome use of immunosuppressive drug. These data demonstrate the feasibility and efficacy of fresh or microencapsulated hepatocyte transplantation in the spleen in carbon tetrachloride induced hepatotoxicity in hamster model.

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