6. Summary & Conclusion

The present work was performed to investigate and assess bone marrow cells transplantation and low dose (0.5 Gy) gamma irradiation effects against carbon tetrachloride-induced histological, histochemical, biochemical and cytogenetical changes in male rat liver cells.

Animals were classified into 6 groups as following:

1. **Control normal group.**
2. **Carbon tetrachloride administrated group.**
3. **Irradiated groups.**
4. **Irradiated and carbon tetrachloride administrated group**s.
5. **Bone marrow treated groups.**

All these groups of rats were sacrificed at one appropriate conditions and circumstances and the results of the present experiment revealed the following observations:

A) <u>Histopathological changes:</u>

- A remarkable changes were depicted in groups administrated carbon tetrachloride; these changes could be illustrated by cytoplasmic degeneration and vacuolation with inflammatory cellular infiltration and necrotic areas. Fatty changes, polyploidy with hemorrhagic infiltration, marked increased amounts of collagen fibers, hypertrophy of the hepatocytes with nuclear chromatin fragmentation and vacuolated lipoma. These changes appeared more conspicuous eight weeks after administration. In bone marrow cells

treated groups there was a remarkable curative effect where the hepatocytes showed marked improvement illustrated by hepatocytes regeneration as most of the hepatocytes nuclei restored their normal appearance, while others showed that the process of degeneration proceeded to necrotic areas, with a significant decrease in the collagen fibers in many areas.

- In low dose (0.5 Gy) gamma irradiated group some pathological changes were depicted indicated by loss of the normal architecture of the hepatocytes with areas of necrosis, an increase in the collagen fibers deposition around the blood vessels and in between the hepatocytes, portal fibrosis and cytoplasmic degeneration with dilatation of the blood sinusoids four weeks post exposure, while eight weeks later, hepatocytes appeared to some extent retained their normal appearance with more or less healthy nuclei and decreased collagen fibers indicating therapeutic effect of low dose irradiation. In bone marrow cells treated group there was more curative effect where the hepatocytes seemed to be normal and healthier.

- Both carbon tetrachloride and low dose gamma radiation application to the rats for 8 weeks showed curative effect as the hepatocytes appeared nearly normal and healthy. Also, bone marrow cells transplantation to both carbon tetrachloride and low dose gamma irradiated rats showed nearly normal appearance of the hepatocytes at the same level of the above mentioned group except for a sign of fibrosis indicated by some collagen fibers depositions around the blood vessels.

- Bone marrow transplantation to normal rats showed no pathological changes.

B) Histochemical Observations:

**** Mucopolysaccharide Content:***

- Remarkable depletion of mucopolysaccharide content was observed in carbon tetrachloride administrated groups which is more prominent at those administrated for eight weeks. On the other hand the rats treated with bone marrow cells showed a detectable increase in the mucopolysaccharide content.

- Gradual restoration of the mucopolysaccharide content was noted in low dose (0.5 Gy) gamma irradiated group eight weeks post irradiation while bone marrow cells transplantation to it showed more or less normal mucopolysaccharide content throughout the hepatocytes.

- An increase in the hepatocytes mucopolysaccharide content was noted in both carbon tetrachloride and low dose gamma irradiated group eight weeks after administration. Bone marrow cells transplantation to the rats of such group showed complete restoration of the mucopolysaccharide particles throughout the hepatocytes.

- Bone marrow transplantation to normal rats showed normal mucopolysaccharide content in a degree similar to that of the control.

**** Deoxyribonucleic Acid (DNA):***

- A decrease in the DNA content of the hepatocytes was noted in carbon tetrachloride administrated group for eight weeks. On the other hand

bone marrow cells transplantation to the rats of such group showed more or less normal nuclear DNA content with an increase in the nuclear size of some hepatocytes.

- Marked increase in the DNA content was noted in low dose (0.5 Gy) gamma irradiated group especially eight weeks post irradiation. Bone marrow transplantation to the rats of such group showed that hepatocytes nuclei retained their normal DNA appearance with the appearance of normal mitotic figures.

- An improvement of the nuclear DNA content was noted in both carbon tetrachloride and low dose gamma irradiated group eight weeks post application. Bone marrow cells transplantation to the rats of such group showed nearly normal appearance of the nuclear DNA content.

- Bone marrow cells transplantation to normal rats showed no DNA containing particles changes throughout the hepatocytes.

C) Ultra structural Alterations:

- Fine structural changes were clearly observed in the rats group administrated with carbon tetrachloride which is more prominent eight weeks post administration where the hepatocytes showed ill defined cytoplasm with dilated blood sinusoids, mitochondria were electron dense, malformed and swollen with disruption of inner and outer membranes surrounded by fragmented rough endoplasmic reticulum with detachment of ribosomes. Hepatocytes appeared infiltrated with red blood cells and necrotic areas with deposition of melanin pigments and increased amounts of mucopolysaccharide particles, vacuoles and lipoma. Excessive amounts of collagen fibers

were also depicted. Some hepatocytes showed pyknotic nuclei with abnormal heterochromatin distribution that appeared gathered at the perimeter with condensed nucleoli. Bone marrow transplantation to this group showed that the hepatocytes seemed to be normal; the nuclei looked to be healthy with normal euchromatin and heterochromatin distribution.

- Rats irradiated with 0.5 Gy of gamma radiation showed moderate to complete curative effect where most of the hepatocytes appeared ultrtastructurally normal. Bone marrow cells transplantation to the rats of this group showed that the hepatocytes cytoplasm appeared more or less normal with normal collagen fibers in the space of Disse. Nuclei appeared containing well distributed euchromatin with marked heterochromatin attached to the inner nuclear envelope.

- Slight improvement of the hepatocytes was noted in both CCl_4 and low dose gamma radiation administrated rats where mitochondria appeared polymorphic, some healthy and other ruptured endoplasmic reticulum still evident with free ribosomes, mucopolysaccharide rosettes and melanin droplets scattered through out the cytoplasm were also noted. Blood sinusoid appeared dilated with more or less normal appearance of the nuclei. Bone marrow cells transplantation to the rats of this group showed that the hepatocytes appeared more or less normal with few collagen fibers depicted beside the blood sinusoid and normal shaped nuclei.

- Bone marrow cells transplantation to normal rats showed normal appearance of the hepatocytes indicating no pathological changes after bone marrow cells transplantation.

D) DNA Cytometry:

- CCl_4 administrated rats group for four weeks & eight weeks showed an increase in the hypoploid cells indicating marked decrease in the DNA content. Bone marrow transplantation to the rats of this group showed more or less normal DNA value.

- Low dose (0.5 Gy) gamma irradiated rat group for four & eight weeks showed an increase in the DNA values. On the other hand bone marrow cells transplantation to the rats of this group showed DNA value more or less comparable to the control values.

- An improvement of the DNA value was noted in both CCl_4 and low dose (0.5 Gy) gamma irradiated rats group eight weeks post administration. Bone marrow cells transplantation to the rats of this group showed restoration of the DNA values that appeared more or less comparable to that of the control values.

- Bone marrow cells transplantation to normal rats showed DNA value more or less similar to the of the control rats.

E) Biochemical alterations:

**** Alanine transaminase (ALT):***

- Carbon tetrachloride administration to the rats for 4 & 8 weeks showed a significant increase in serum levels of ALT enzyme as compared to their corresponding control value. Rats exposed to fractionated low dose 0.5 Gy of gamma radiation for 4 & 8 weeks showed decrease in the ALT level. Rats exposed to low dose 0.5 Gy gamma radiation in combination with CCl_4 showed a significant decrease in the ALT level 4 weeks post administration while a

significant increase was noted 8 weeks latter. Administration of bone marrow ameliorated the damage induced by CCl_4 and low dose radiation in all examined rat groups.

**** Aspartate transaminase (AST):***

- Carbon tetrachloride administration to the rats for 4 & 8 weeks showed a significant increase in serum levels of AST enzyme as compared to their corresponding control value. Rats exposed to low dose 0.5 Gy of gamma radiation for 8 weeks showed a significant increase in the AST level. Rats exposed to 0.5 Gy gamma radiation in combination with CCl_4 showed a significant increase in the AST level 8 weeks post its administration. Injection of bone marrow cells elevated the increases in serum AST level in all examined rat groups.

**** Total Protein Level:***

- CCl_4 administration showed a decrease in the rat serum total protein level 4 & 8 weeks post administration. Whole body gamma irradiation (0.5 Gy) of rats caused a significant increase in the total protein level 4 & 8 weeks respectively. Rats exposed to 0.5 Gy gamma radiation in combination with CCl_4 showed a significant increase in the total protein level 4 & 8 weeks respectively too. Bone marrow cells administration ameliorated the damage induced by CCl_4 and low dose gamma radiation in all examined rat groups.

**** Lipid Peroxidation Level:***

- Carbon tetrachloride administrated rats for 4 & 8 weeks showed a significant increase in the lipid peroxidation levels. Rats exposed to 0.5 Gy gamma radiations exhibited a significant decrease 4 weeks post exposure while 8 weeks post exposure showed a significant

increase in the lipid peroxidation levels. Administration of both CCl_4 and gamma radiation exposure revealed a significant increase in the lipid peroxidation levels 4 & 8 weeks post administration. Bone marrow cells transplantation ameliorated the lipid peroxidation changes in all treated groups.

**** Hydroxyproline Level**:*

- There is a significant increase in the hydroxyproline level of the groups injected with carbon tetrachloride 4 & 8 weeks respectively as compared to control. In low dose (0.5 Gy) gamma radiation group a decrease in the hydroxyproline level is noted 4 & 8 weeks respectively. Rats exposed to 0.5 Gy gamma radiations in combination with CCl_4 showed a decrease in the hydroxyproline level 4 & 8 week respectively post administration. Injection of bone marrow cells ameliorated the hydroxyproline levels damage induced in all transplanted rat groups.

**** Glutathione Levels (GSH):***

- A significant decrease in the glutathione level was noted in the groups injected with carbon tetrachloride for 4 & 8 weeks respectively as compared to control. In low dose (0.5 Gy) gamma radiation group a significant increase in glutathione level was noted 8 weeks post radiation exposure. Rats exposed to 0.5 Gy gamma radiations in combination with CCl_4 showed a significant decrease in the glutathione level 4 & 8 weeks respectively post administration. Injection of bone marrow cells ameliorated the glutathione levels damage induced in all transplanted rat groups.

F) Cytogenetic alterations:

- More chromosomal aberrations were detected in carbon tetrachloride administrated rats. The highest types of chromosomal anomalies were sticky, and deletion followed by centromeric attenuation, centric fusion, fragments, end to end associations, gaps and breaks. On the other hand carbon tetrachloride administration induced a decrease in the mitotic activity of bone marrow cells. Bone marrow transplantation to the rats of these groups decreased the number of sticky, deletion, fragment, centromeric attenuation, centric fusion, end to end associations, gaps and breaks and with an increase in the mitotic index of the bone marrow cells.

- Low dose (0.5 Gy) gamma irradiation of rats showed an increase in the number of chromosomal aberrations including sticky, deletion, chromatid fragment, centromeric attenuation, centric fusion, end to end association, gap and break. A decrease in the mitotic index was also noted in the rats of such groups. Bone marrow transplantation to the rats of these groups decreased the number of chromosomal aberrations especially sticky and deletion followed by fragment, centromeric attenuation, centric fusion, end to end associations, gaps and breaks with an increase in the mitotic index.

- Administration of both carbon tetrachloride and low dose gamma radiation to the rats increased the chromosomal abnormalities which were clearly observed in increasing the number of sticky, deletion, centromeric attenuation, centric fusion, end to end association, gap and break. A decrease in the mitotic index was also noted. Bone marrow transplantation to the rats of these groups decreased the number of chromosomal aberrations with an increase in the mitotic index.

From these results it can be concluded that, the transplantation of bone marrow cells and exposure to low dose gamma radiation showed marked therapeutic role in liver cells which may be due to:

- Transdifferentiation of the transplanted bone marrow cells into new hepatocytes.

- The bystander effect where the transplanted bone marrow cells stimulated the resident hepatocytes proliferation through factors secreted by the bone marrow derived cells.

- Transplantation of bone marrow cells may induce an indirect mechanism for the amelioration of liver fibrosis, possibly via stellate cells or Kupffer cells where Kupffer cells were able to produce proteases that can digest collagen fibers.

- Transplanted bone marrow cells may affect activated stellate cells by reducing their number e.g., by leading them to apoptosis which could be another mechanism for liver regeneration.

- Transplantation of bone marrow cells increases the mitotic index of the bone marrow cells and decreases the chromosomal abnormalities.

- Chronic low doses of gamma radiations stimulate prevention and repair of DNA damage and the immune system, leading to the beneficial effects of decreased mortality in general and decreased cancer mortality specifically.

- Chronic low dose gamma radiation exposure may induce two principal types of adaptive protection, one is to prevent and repair DNA damage and in doing so to keep cells alive and functioning properly. The other is to remove damaged cells from tissue by inducing apoptosis.

- Low doses of gamma rays induce defense such as detoxification of reactive oxygen species and activate the immune response.

- The induction of endogenous glutathione after exposure to low-dose γ-irradiation may be beneficial in protecting the cells from reactive oxygen species (ROS) induced oxidative stress.