**Summary**

The present study aimed to elucidate the therapeutic effects of mesenchymal stem cells derived from the bone marrow and adipose tissue of rats against toxic effects of busulfan on the male gonads of experimental rats using histological analysis. In this study we used Thirty-five male wistar rats (8-12 weeks old, average weight 200-220 g) and 28 adult female wistar rats . The recipient animals were kept under standard conditions, at 22–25 °C with a 12-h light/dark cycle, with food and water freely available throughout the study. Male rats were randomly divided into four groups. Rats in group I (control group) were equally subdivided into subgroups Ia, Ib; 5 rats each. Rats in subgroups Ia were injected with a single intra-peritoneal dose of 0.1 ml PBS/100 gm body weight and after 8 weeks 3rats of the group were sacrificed for histological examination while the other 2 rats were left to be matted with the 4 females of group A. Subgroup Ib served as donors for stem cells obtained from their bone marrow and adipose tissue. Group II rats (busulfan treated group) (n=5) were subjected to intra-peritoneal injection of 2 doses of busulfan with 3 weeks interval the first dose of 15mg/kg body weight dissolved in 0.1 ml PBS the second dose of 30mg/kg body weight and 3rats of the group were sacrificed after 5 weeks from the second dose for histological examination of testes while the other 2 rats were matted with the 4 females of group B. Group III (BM-MSCs treated group) (n=10) were treated by busulfan in the same manner as in group II and after five weeks from the second busulfan injection 100 μl of BM-MSCs mixture (2.5x106 cells) in 100 µl PBS was injected intra-testicular and 5 rats of this group were sacrificed 12 weeks after stem cells injection and the other 5 rats were left to be matted with female group C.

Group IV rats (AT-MSCs treated group) (n=10) were treated by busulfan as in group II and after 5 weeks from the second busulfan injection, 100 μl of AT-MSCs mixture (2.5x106 cells) in 100µl PBS was injected intra-testicular, 5 rats of the group were sacrificed 12 weeks after stem cells injection while the other 5 rats were left to be matted with females group D.

The rats were sacrificed and testes were dissected, examined and prepared for electron microscopic examination, light microscopic using Hx.& E and PCNA &CD105 immunostaining and morphometric studies.

We assessed the effect of transplanted cells on spermatogenesis via macroscopic and microscopic examination of testes .

By naked eye examination of the studied testes, the busulfan injected group showed atrophied testes, while MSCs groups showed normal sized testis.

Stem cell engraftment was detected using fluorescent microscopic (Leica) examination, PKH26-stained testis tissue emitted pink fluorescence that indicated homing of stem cells into the seminiferous tubules.

The present work demonstrated that Haematoxylin and eosin-stained sections of control rats testis showed normal morphology. It was covered by thin connective tissue capsule. The seminiferous tubules were densely packed and lined with multiple layers of spermatogenic cells with little interstitium-containing clusters of interstitial cells and blood vessels

In Busulfan treated group, busulfan produced variable histological changes in the testes of the rats. These changes appeared as loss of normal architecture of the seminiferous tubules, markedly reduction in the all types of spermatogenic cells which caused shrinkage of the tubules and subsequently dilatation of intercellular spaces, detachment of spermatogenic cells from the irregular and thickened basal lamina. Dilated congested blood vessels and vacuoles in the interstitial tissues were seen. Busulfan group also showed decreased expression of PCNA. These findings were supported by morphometric results and statistical analysis which showed that the mean values of seminiferous tubule diameter, cross sectional area, cellular diameter, spermatogenesis index and area percent of PCNA immunoreactivity were highly significantly decreased in busulfan treated group as compared to control group (P<0.01), while the mean values of luminal diameter and luminal area were highly significantly increased in busulfan treated group as compared to control group (P<0.01).

***BM-MSCs treated rats showed*** restoration of normal architecture. In this study it was observed that testes of the rats of group III showed minimal histological changes as compared to those of group I.BM-MSCs treated group showed increased expression of PCNA. These findings were supported by morphometric results and statistical analysis which showed that the mean values of seminiferous tubule cellular diameter, spermatogenesis index and the area percent of PCNA immunoreactivity were markedly improved with no significant difference when compared to control group (P>0.05) and they were highly significantly increased when compared to busulfan treated group (P<0.01). while the mean values of luminal diameter and luminal area were highly significantly decreased in BM group as compared to busulfan group (P<0.01) but they decreased with no significant difference as compared to control group (P>0.05).

**AT-MSCs treated group** also showedrestoration of normal architecture. minimal histological changes as compared to those of group I. Also histopathological effects of busulfan administration on the testicular structure as well as the reproductive function have been improved with MSCs administration. AT-MSCs group showed increased expression of PCNA. These findings were supported by morphometric results and statistical analysis which showed that the mean value of spermatogenesis index was markedly improved with no significant difference when compared to control group (P>0.05) and it was highly significantly increased when compared to busulfan treated group (P≤0.01) and the area percent of PCNA immunoreactivity was significantly increased when comparedto busulfan treated group (P<0.05). BM & AT-MSC treated testicular tissue have shown positive cell membranous reaction for CD105 in the form of brown pigmentation of cell membrane and cytoplasm of spermatogenic cells which means that transplanted BM & AT-MSCs have differentiated into these spermatogenic cells as CD105 characterizes MSCs.

**Mating the Rats:** Female rats (𝑛 = 28) were mated with male rats (𝑛 = 14) overnight (2 male of control group with 4 female group A), ( 2 male of busulfan treated group with 4 female group B), ( 5 male of BM-MSCs transplantation with 10 female group C) and (5 male of AT-MSCs transplantation with 10 female group D). Every male rat was cohabitated with two female rats in polycarbonate cages until evidence of mating, mating was assessed by the presence of a vaginal plug on the following morning*.* On the 20th day of gestation. i.e. 12-24 hours before the expected day of delivery to prevent the mothers from devouring any damaged offspring. 2 female rats of each group, both the experimental and control were anaesthetized by inhalation of ether. The anterior abdominal wall was incised and the uterus was photographed .The uterine horns were carefully inspected. The number of implantation sites, the resorption sites, live and dead fetuses were counted. The implantation sites indicate the original sites of embryos irrespective of whether they have been survived or have undergone resorption. A resorption site was indicated by a dark brown blood spot and it refers to early post implantation death. However, late post-implantation death appears as a large blood clot attached to the uterine wall at the site of implantation. Then the offspring of other females were examined morphologically. We found in the female rat matted with control rats, normal pregnant uterus has shown normal distribution of fetuses within the uterine horns with 10 fetuses within the uterus.The other one has shownnormal pregnant uterus with normal distribution of fetuses within the uterine horns with 8 fetuses within the uterus. In the female rat matted with males treated by busulfan, only 2 of the 4 females become pregnant, one uterus has shown dark brown spots indicating early post-implantation loss with 5 fetuses. The other one has shown large blood clots indicating a site of late post-implantation resorption with 2 fetuses within the uterus. In the female rat matted with males treated by BM-MSCs , normal pregnant uterus has shown normal distribution of fetuses within the uterine horns with 8 fetuses. And Offspring of females matted with BM-MSCs treated group have shown normal development and growth. In the female rat matted with males treated by AT-MSCs , normal distribution of fetuses within the uterine horns has been observed which are 6 fetuses. And Offspring of females matted with AT-MSCs treated group have shown normal development and growth.