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Original article

Therapeutic and Protective Effects of Undifferentiated Stem Cells Against Paraquat-Induced Liver Inflammation in Rats

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Abstract
Paraquat (PQ) is a highly toxic herbicide that can induce oxidative stress, inflammation, and tissue injury, including hepatic injury. Mesenchymal stem/stromal cells have attracted considerable interest because of their immunomodulatory and tissue-repair properties. A total of 65 adult male albino rats were divided into four groups: control (n=8), PQ-treated (n=11), protective UDSC-treated (n=16), and therapeutic UDSC-treated (n=30). The PQ-treated group received two successive intraperitoneal doses of PQ at 7.5 mg/kg during Weeks 3 and 4. The protective group received UDSCs intravenously during Week 3, followed one hour later by a single PQ dose of 7.5 mg/kg. The therapeutic group received two successive PQ doses during Weeks 3 and 4, followed by intravenous UDSC administration at a dose of 2,000 cells/g body weight during Week 6, approximately three weeks after the appearance of inflammatory signs. Liver tissues were subsequently examined histologically using hematoxylin and eosin staining. Inflammatory-cell count was used as a quantitative histopathological parameter, and differences among groups were evaluated using one-way analysis of variance (ANOVA). Paraquat exposure was associated with increased inflammatory-cell infiltration and vascular congestion compared with the control group. UDSC administration was associated with improvement in hepatic histopathological features in both treatment groups. An estimated 25% reduction in inflammatory-cell changes was observed in the protective group, whereas the therapeutic group showed an estimated 45% reduction. The therapeutic group demonstrated a greater apparent reduction in inflammatory-cell infiltration than the protective group. UDSC administration was associated with attenuation of paraquat-associated hepatic inflammatory changes in rats. The findings suggest that UDSCs may have both protective and therapeutic potential, with a greater apparent histopathological improvement observed following therapeutic administration after the development of inflammatory signs. Further studies using standardized histopathological scoring, biochemical liver-function markers, molecular inflammatory and oxidative-stress parameters, and comprehensive statistical analyses are required to confirm these findings and clarify the underlying mechanisms
Keywords. Paraquat, Liver Inflammation, Mesenchymal Stem Cells, Undifferentiated Stem Cells, Hepatic Injury.

Introduction

Paraquat is a widely recognized herbicidal compound with substantial toxicological potential. Exposure to paraquat can induce oxidative and cellular stress and may result in tissue injury. The liver is particularly relevant to toxicological studies because of its central role in xenobiotic metabolism and its complex interactions with resident and recruited inflammatory cells. Liver inflammation is a complex response to cellular and tissue injury. Hepatic inflammatory responses involve resident macrophages (Kupffer cells) and recruited immune cells, including macrophages, T lymphocytes, neutrophils, natural killer cells, and other leukocyte populations. These cells can release cytokines, chemokines, reactive oxygen species, and other mediators that influence hepatocyte survival, stellate-cell activation, extracellular-matrix deposition, and tissue repair. Previous studies have described relationships among hepatic injury, inflammatory signaling, apoptosis, and fibrosis.

Inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), and interferon-gamma (IFN-γ) may contribute to the progression or resolution of hepatic injury, depending on the context and stage of the response [1,2,3]. Whole-organ liver transplantation remains an established treatment for end-stage liver disease; however, its application is limited by donor-organ availability, surgical risks, immunological complications, and cost. These limitations have stimulated interest in regenerative approaches, including stem-cell-based therapies. Stem cells are characterized by their capacity for self-renewal and differentiation. Mesenchymal stem/stromal cells (MSCs), also referred to in the literature as marrow stromal cells or mesenchymal progenitor cells, have been investigated because of their multilineage potential and their immunomodulatory and paracrine effects. Experimental studies have reported that MSCs can influence inflammation, apoptosis, angiogenesis, tissue repair, and fibrosis [6,7]. MSCs have also been investigated as a potential therapeutic strategy for liver injury. Their proposed benefits may involve paracrine signaling and secretion of trophic factors rather than direct replacement of damaged hepatocytes alone. However, the biological effects depend on the cell source, cell characterization, dose, route of administration, timing, and experimental model [11.13].

The present study investigated histological liver changes following paraquat administration in rats and evaluated whether intravenous administration of undifferentiated stem cells was associated with protective effects when given before paraquat exposure and therapeutic effects when given after paraquat-induced injury. The study also aimed to explore the possible role of stem-cell treatment in ameliorating the degenerative and inflammatory hepatic changes associated with paraquat exposure.

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Materials and Methods

Chemicals and Reagents

Paraquat dichloride (PQ; 1,1'-dimethyl-4,4'-bipyridinium dichloride hydrate; Sigma-Aldrich), DPX mounting medium, 10% normal serum, 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS), 0.3% hydrogen peroxide, Mayer's hematoxylin, and formalin-alcohol-glacial acetic acid were reported in the original manuscript.

Experimental Animals

A total of 65 adult male albino rats, 10–12 weeks of age and weighing approximately 250–350 g, were obtained from the Central Animal House, Faculty of Medicine, Kasr El Einy, Cairo University. The original manuscript states that animal procedures followed institutional and international guidance, including the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the National Institutes of Health (NIH) protocol. The animals were acclimatized for three weeks and housed in well-ventilated cages under standard laboratory conditions, including a temperature of approximately 25 °C, relative humidity of 60–70%, and a 12-h light/12-h dark cycle. Standard commercial pellet diet and tap water were provided ad libitum.

Experimental Design and Treatment Groups

A total of 65 adult male albino rats were divided into four experimental groups: Group I (control, n=8), Group II (paraquat-treated, n=11), Group III (protective UDSC-treated, n=16), and Group IV (therapeutic UDSC-treated, n=30). Group I served as the control group and received 0.9% normal saline intraperitoneally (IP). Group II received two successive IP doses of paraquat (PQ), each at 7.5 mg/kg, during Weeks 3 and 4.

Group III received undifferentiated stem cells (UDSCs) intravenously (IV) during Week 3 as a protective intervention. One hour after UDSC administration, the animals received a single IP dose of PQ at 7.5 mg/kg. No additional intervention was administered to this group during Week 4. This schedule was designed to evaluate the potential protective effect of UDSCs administered before PQ exposure.

Group IV received two successive IP doses of PQ, each at 7.5 mg/kg, during Weeks 3 and 4. Inflammatory signs were observed during Week 3 following PQ exposure. During Week 6, approximately three weeks after the appearance of inflammatory signs, UDSCs were administered intravenously at a dose of 2,000 cells/g body weight (BW). This schedule was designed to evaluate the potential therapeutic effect of UDSCs after the development of PQ-associated hepatic inflammatory changes. The therapeutic group was sacrificed during Week 9 for histopathological examination.

Experimental Timeline

Following a 20-day acclimatization period, experimental interventions were initiated during Week 3. The control group received 0.9% normal saline intraperitoneally. The paraquat-treated group received the first dose of PQ (7.5 mg/kg, IP) during Week 3 and a second dose of 7.5 mg/kg during Week 4. The protective group received UDSCs intravenously during Week 3, followed one hour later by a single IP dose of PQ (7.5 mg/kg), with no additional intervention during Week 4. The therapeutic group received PQ at 7.5 mg/kg IP during Weeks 3 and 4. Inflammatory signs were observed during Week 3. UDSCs were administered intravenously to the therapeutic group during Week 6, approximately three weeks after the appearance of inflammatory signs, at a dose of 2,000 cells/g BW. Thus, UDSCs were administered as a protective intervention in Group III and as a therapeutic intervention in Group IV. The therapeutic group was sacrificed during Week 9, and liver tissues were collected for histopathological examination

Table 1. Showing the stem cell preparation protocol, mainly based on frequent medium changes in primary culture and reducing the trypsinization time

Time point	Group I – Control	Group II – Paraquat	Group III – Protective UDSC	Group IV – Therapeutic UDSC
Adaptation period	20 days	20 days	20 days	20 days
Week 3	0.9% saline IP	PQ 7.5 mg/kg IP – first dose	UDSCs IV → 1 h → PQ 7.5 mg/kg IP	PQ 7.5 mg/kg IP – first dose
Week 4	No intervention	PQ 7.5 mg/kg IP – second dose	No intervention	PQ 7.5 mg/kg IP – second dose
Week 6	No intervention	No intervention	No intervention	UDSCs IV (2,000 cells/g BW)
Week 9	—	—	—	Sacrifice and histopathological examination

Isolation and Culture of Mesenchymal Stem Cells

The original manuscript reports that MSCs were isolated from bone marrow aspirates obtained from the tibial and femoral marrow compartments and cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (FBS). Cultures were maintained at 37 °C in a humidified incubator containing 5% CO_2. Non-adherent cells were removed,

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and fresh medium was added. When cultures approached confluence, cells were treated with 0.25% trypsin containing 0.02% EDTA for approximately 2 min at room temperature. The manuscript states that a purified MSC population could be obtained approximately three weeks after initiation of culture. The original text also contains a statement concerning a growth factor used for neurogenic differentiation. Because the present study concerns hepatic injury and undifferentiated cells, the identity and purpose of this growth factor are unclear and should be verified before publication. The manuscript does not provide sufficient phenotypic characterization markers (for example, surface-marker expression) to independently confirm MSC identity.

Histopathological Assessment

Liver tissues were collected according to the experimental schedule and processed for histopathological examination. Sections were stained with hematoxylin and eosin (H&E) and examined microscopically by a specialist in pathology and histopathology. The assessment focused on the general hepatic architecture and inflammatory changes, particularly inflammatory-cell infiltration. The inflammatory cell count was used as a quantitative histopathological parameter for comparison among the experimental groups.

Statistical Analysis

Histopathological inflammatory cell count data were expressed as mean ± standard error of the mean (SEM). Differences among the experimental groups were evaluated using one-way analysis of variance (ANOVA). A P-value of <0.05 was considered statistically significant. Statistical analysis was performed based on the inflammatory cell count obtained from the histopathological assessment.

Results

Histological Findings

The control liver sections showed preserved hepatic architecture and were described as having normal histological features. In contrast, liver sections from paraquat-treated rats showed inflammatory-cell infiltration and congestion. These findings are consistent with hepatic inflammatory injury in the experimental model. In the therapeutic group, liver sections obtained after UDSC administration were described as showing substantially improved histological architecture, with features more closely resembling those observed in the control group. Similarly, liver sections from the protective group, in which UDSCs were administered before paraquat exposure, were described as showing relatively preserved histological features.

Inflammatory Cell Count

Table 2 and Figure 6 show the effect of paraquat (PQ) exposure and UDSC administration on inflammatory cell counts in rat liver tissue. Compared with the control group, the PQ-treated group showed an estimated 52% increase in inflammatory cell count, indicating a marked inflammatory response following paraquat exposure. In contrast, UDSC administration was associated with a reduction in inflammatory cell counts in both treatment groups, with estimated decreases of 25% in the protective group and 45% in the therapeutic group. The greater reduction observed in the therapeutic group suggests a more pronounced improvement in the inflammatory histopathological response following UDSC administration after the development of inflammatory signs. Statistical analysis was performed using one-way analysis of variance (ANOVA), with statistical significance considered at $P < 0.05$.

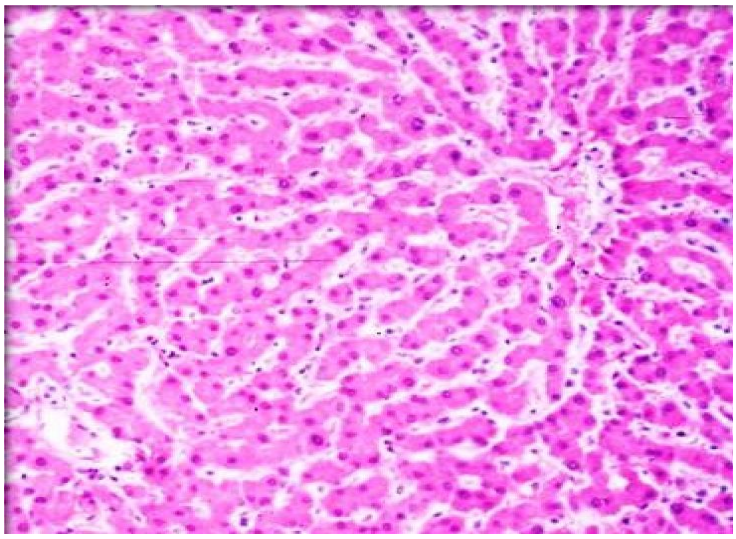

Figure 1. Representative H&E-stained sections of rat liver. Control liver showing preserved normal histological architecture

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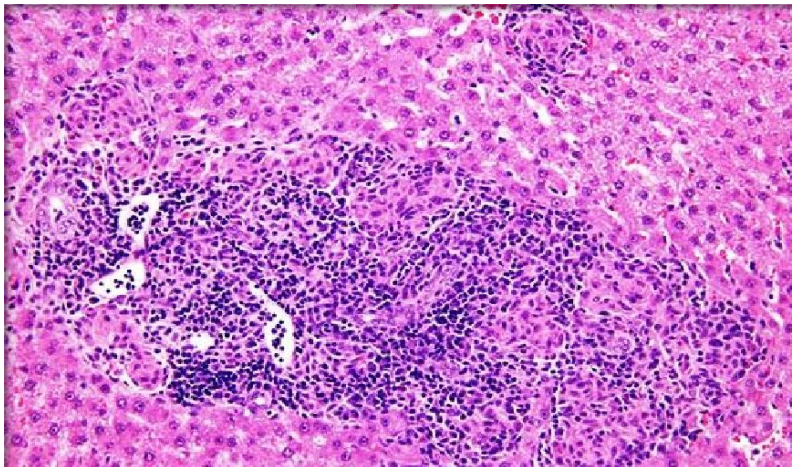

Figure 2. Showing the liver of rats treated with 15 mg of PQ, showing areas of inflammatory cell invasion (H&E 100 X)

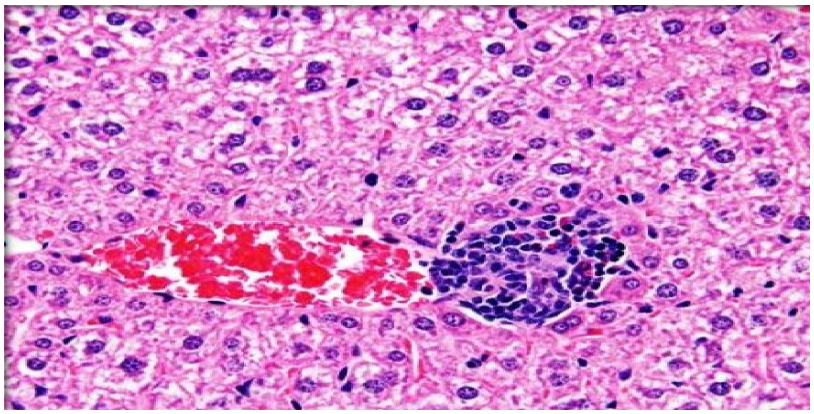

Figure 3. Showing the Magnified part of the previous section treated with two successive doses of PQ, showing areas of inflammatory cell invasion. (pointed arrow) and congestion (arrow)

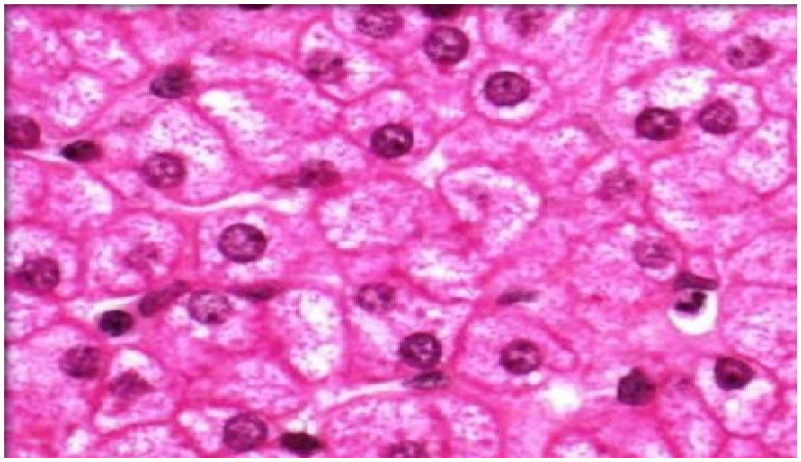

Figure 4. Schematic representation of the experimental timeline and treatment schedule. In the protective group, UDSCs were administered intravenously during Week 3, followed one hour later by a single paraquat dose of 7.5 mg/kg. In the therapeutic group, paraquat was administered at 7.5 mg/kg during Weeks 3 and 4. Inflammatory signs were observed during Week 3, and UDSCs were administered intravenously during Week 6, three weeks after the appearance of inflammatory signs. The therapeutic animals were sacrificed during Week 9.

Table 2. Effect of paraquat exposure and UDSC administration on inflammatory cell counts in rat liver tissue.

Parameter	Control	PQ	Protective UDSC	Therapeutic UDSC
Inflammatory cell count	28.43 ± 2.091	34.14 ± 2.219	24.86 ± 2.444	18.71 ± 1.748
% of change	—	52% ↑	25% ↓	45% ↓

Data are presented as mean ± SEM. Statistical significance was determined

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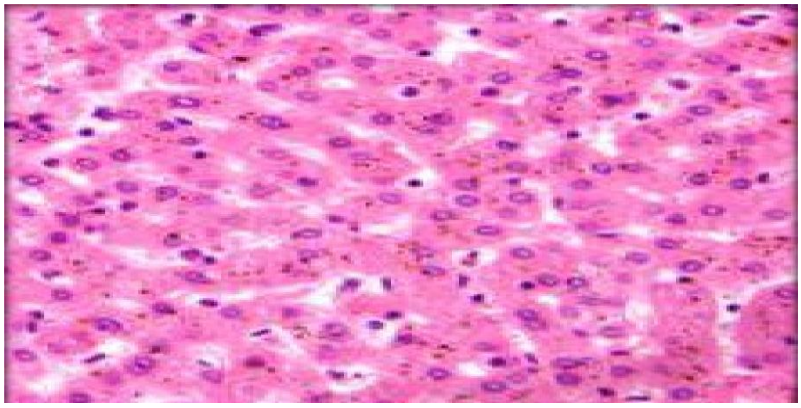

Figure 5. Showing a section of rat liver injected with UDSCs (2000 cells/1g BW) before one hour of PQ injection, showing that normal histology looks more or less similar to that of the control (H&E 400X)

using one-way ANOVA. P < 0.05 was considered statistically significant.

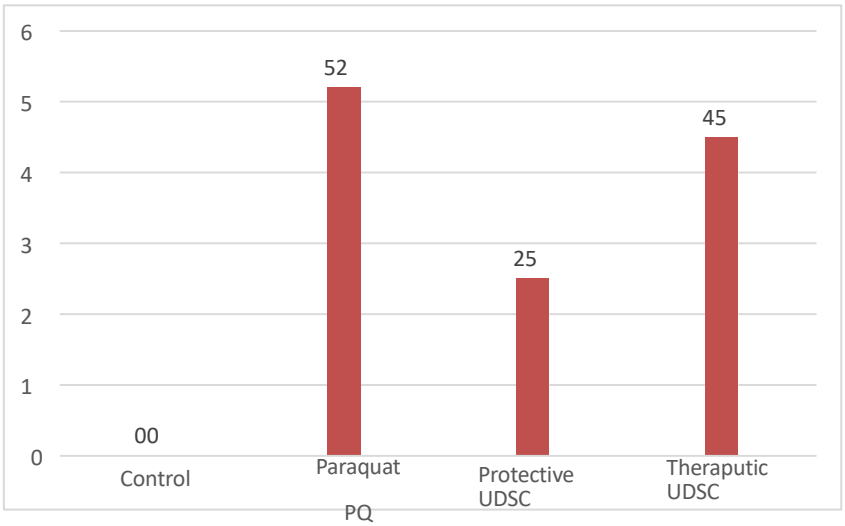

Figure 6. Comparative histopathological assessment of rat liver following paraquat exposure and UDSC administration.

Discussion

Paraquat (PQ) exposure in the present study was associated with clear hepatic inflammatory and histopathological changes, particularly inflammatory-cell infiltration and vascular congestion. The higher inflammatory-cell count observed in the PQ-treated group compared with the control group indicates the development of a hepatic inflammatory response following PQ exposure. The liver contains a complex network of immune cells, including Kupffer cells and recruited inflammatory cells, which participate in both hepatic injury and tissue repair [1,3,4]. Previous experimental studies have also reported that paraquat-induced hepatotoxicity is associated with oxidative stress, cellular damage, and inflammatory responses [15,16].

The reduction in inflammatory-cell infiltration observed following UDSC administration suggests that these cells may have a modulatory effect on the hepatic inflammatory response. In the protective group, UDSCs were administered before PQ exposure, and an estimated 25% reduction in inflammatory-cell changes was observed. This finding suggests that administration of UDSCs before toxic exposure may help limit the inflammatory response associated with PQ-induced hepatic injury. The ability of mesenchymal stem/stromal cells to participate in tissue repair and modulate inflammatory responses has been reported in several experimental studies [7,10,11].

A greater estimated reduction in inflammatory-cell changes was observed in the therapeutic group (45%) compared with the protective group (25%). In this group, UDSCs were administered during Week 6, approximately three weeks after the appearance of inflammatory signs. The improvement observed after treatment suggests that UDSCs may also have a therapeutic effect on an already established inflammatory response. This may be related to the immunomodulatory and paracrine properties of MSCs rather than direct replacement of damaged cells. MSCs can release various soluble factors that influence immune cells and the local tissue environment, thereby supporting tissue repair [14,17,18].

The greater apparent improvement in the therapeutic group is particularly interesting because the cells were administered after the development of inflammatory changes. This finding suggests that UDSCs may remain effective even when hepatic inflammation has already been established. However, the present study cannot determine the precise mechanism responsible for this difference. The observed histological improvement may involve modulation of inflammatory cells, reduction of oxidative stress, and stimulation of endogenous repair processes. Further biochemical and molecular investigations would be required to distinguish among these possible mechanisms.

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The findings should also be interpreted within the limitations of the present study. The main quantitative histopathological parameter assessed was the **inflammatory-cell count**, together with microscopic evaluation of hepatic architecture and vascular congestion. Although one-way ANOVA was used to evaluate differences among the experimental groups, the study did not include a comprehensive panel of biochemical or molecular markers of liver injury and inflammation. In addition, further characterization of the administered UDSCs would strengthen the interpretation and reproducibility of the findings. Overall, the present results suggest that UDSC administration was associated with a reduction in paraquat-related hepatic inflammatory changes in both the protective and therapeutic groups. The greater apparent reduction in the therapeutic group may indicate a beneficial effect of UDSCs when administered after the development of inflammatory changes. Nevertheless, these findings should be confirmed in future studies using standardized cell characterization, quantitative histopathological assessment, serum liver-function markers, and molecular indicators of inflammation and oxidative stress.

Conclusion

paraquat exposure produced evident hepatic inflammatory changes in rats, characterized mainly by increased inflammatory-cell infiltration and vascular congestion. Administration of undifferentiated stem cells (UDSCs) was associated with improvement in these hepatic changes in both the protective and therapeutic groups. The protective group showed an estimated 25% reduction in inflammatory-cell changes, whereas the therapeutic group showed a greater estimated reduction of 45%. These findings suggest that UDSCs may have both protective and therapeutic potential against paraquat-associated hepatic inflammation, with a greater apparent benefit when administered after the development of inflammatory changes. However, further studies using standardized cell characterization, quantitative histopathological assessment, biochemical liver-function markers, and molecular inflammatory and oxidative-stress parameters are needed to confirm these findings and clarify the underlying mechanisms.

Conflict of interest. Nil

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