EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ZOOLOGY

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ISSN
2090-0759

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

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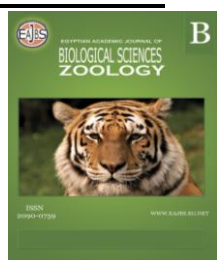
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 14(1) pp: 261-269(2022)*
DOI: 10.21608/EAJBSZ.2022.238371

Egypt. Acad. J. Biolog. Sci., 14(1): 261-269 (2022)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Role of Stem Cells and Erythropoietin on Experimental Acute Hepatic Injury in Rats

Samir A. M. Zaahkouk[1], Mansour, A.[2], Ibrahim, Reda.Hassan[1] and Sawsan El-Shamy[3].

1-Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt
2-Pharmacology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
3- Biology Department, Basic Science, Misr University for Science and Technology, Cairo, Egypt.

E-mail*: hemdanreda5@gmail.com

ARTICLE INFO

Article History
Received:9/4/2022
Accepted:18/5/2022
Available:20/5/2022

Keywords:
Stem Cells, Erythropoietin, Hepatic Injury, rats

ABSTRACT

Erythropoietin (EPO) exhibits non- hematopoietic functions. Besides its hematopoietic functions, EPO has anti-apoptotic, anti-oxidant and anti-inflammatory activities. Tissue-protective EPO effect was confirmed in brain, myocardium, liver, and kidney injury. Mesenchymal stem cells (MSCs) are nonhematopoietic cells. It may be extracted from the bone marrow, dental pulp, adipose tissue, placenta, umbilical cord, or amniotic membrane. Pre-clinical and clinical evidence shows that MSCs derived can heal injured liver tissues, enhance liver functions, and reduce liver fibrosis. **Material and Methods**: Hundred male albino rats were classified into five groups equally. Some biochemical and Immunohistochemical studies of COX-2 were assessed in the liver tissue. **Results:** MSCs and EPO treatment ameliorated the toxic effect of GalN with a significant decrease in the mean level of ALAT, ASAT, and ammonia. The combination between MSCs and EPO showed more effective protection against GalN toxicity.

INTRODUCTION

There are many acute liver injury-inducing agents. Galactosamine (GalN) is considered one of them. The mechanism for Gal-induced liver injury, although poorly understood, seems to be partly related to immunity. Its toxic effects are connected with the UDP sugar insufficiency "UDP-glucose and UDP-galactose", the pro-inflammatory mediators release like TNF-α from Kupffer's cells, and intracellular calcium homeostasis' loss altering uridine-pool in hepatocytes (Izu *et al.,* 2007). These changes affect cell membranes, organelles, and proteins and nucleic acid synthesis. A high GalN dose inhibits the energy metabolism of hepatocytes, damages the enzymes needed for the substrates' delivery to the mitochondria, and modifies the membranes' phospholipid composition (Tawfik *et al.,* 2015).

EPO stimulates the endothelial progenitor cells' proliferation and protraction. It has a pivotal role in the marrow-derived MSCs' proliferation and differentiation (Liu *et al.*, 2011-b).

GalN administration changes the proteoglycans' distribution in the liver and inhibits hepatocyte metabolic activity, as well as modifies the cell membranes' structure and destroys the enzymes needed for the substrates' delivery to the mitochondria (Madro *et al.*, 2009).

MSCs are nonhematopoietic cells. It may be extracted from the bone marrow, adipose tissue, dental pulp, placenta, amniotic membrane, or umbilical cord. *(Favier B et al., 2019).* Pre-clinical and clinical evidence shows that MSCs derived from various sources can heal injured liver tissues, enhance liver functions, and reduce liver fibrosis (Kim. *et al.*,2019).

The MSC's antifibrotic activity may be connected to their migrating and homing efficacy. The local microenvironment also has an effect on the MSC quality. Many in vivo research revealed that only a tiny transplanted cell portion moves to the injury or lesion site, and their antifibrotic activity is minimal (Li *et al.*, 2019).

As a result, understand that enhancing the MSCs' directional migratory ability and increasing the transplanted cells is critical for enhancing the antifibrosis efficiency in chronic injuries, particularly liver fibrosis.

EPO, a glycoprotein hormone generated mostly by the kidneys, promotes the erythrocyte progenitor cells' proliferation and differentiation. EPO protects the kidneys. Nevertheless, accumulated data shows that EPO has a protective role in the nervous system (Merelli *et al.*, 2020).

MATERIALS AND METHODS

In Vivo* Study*:

Hundred male albino rats were classified into five groups equally; ALAT, ASAT, albumin, and ammonia levels were assessed in serum, and an immunohistochemical study of COX-2 was done.

Experimental Design:

A hundred rats were classified into five groups equally:

- **Group I**: the healthy control group.
- **Group II**: they were injected one time with galactosamine GalN (650mg/kg IP) dissolved in saline to cause acute liver toxicity (Bigoniya *et al.*, 2009).
- **Group III**: they were injected one time with MSCs (one million cells/rat IP) [11] (Volarevic *et al.*, 2014) immediately following the GalN injection.
- **Group IV**: they were injected one time with EPO (12 IU/kg IP) modified from [12] (Lipsic *et al.*, 2008) immediately following the GalN injection.
- **Group V:** they were injected one time with MSCs treated with EPO (IP) following the GalN injection (7 days). From the rats' retro-orbital veins, venous blood was taken. By cervical dislocation, the rats were sacrificed.

Immunohistochemistry Study of COX-2:

From each paraffin block, unstained positively charged slides were made for immunostaining by the monoclonal rabbit anti-human antibody (anti-COX-2) and the ultra-vision detection system (HRP/DAB) usage.

RESULTS

Results are illustrated in Figure (1). The results of this work explore the toxic effect of GalN. GalN causes a decrease in albumin levels. While ALAT, ASAT activities, and ammonia levels significantly increase ($P<0.05$). MSCs and EPO alone exhibit a hepatoprotective effect against GalN toxicity with almost close results.

However, pretreatment of MSCs with EPO significantly improves the toxic effect of GalN and significantly decreases ALAT, ASAT activities, and ammonia levels that are elevated by GalN.

Table 1: All measured variables' relationships**.**

	Group I	**Group II**	**Group III**	**Group IV**	**Group V**
Albumin(g/dL)	5.29± 0.45	2.56± 0.46 *	3.28± 0.50 *	3.21± 0.33 *	4.06± 0.56 *#
Ammonia(µg/dL)	11.86± 1.97	74.42± 5.66 *	37.90± 10.06 *#	41.76± 8.17 *#	21.72± 5.10 #$@
ALT(U/L)	27.14± 5.20	91.56± 6.59 *	47.50± 7.27 *#	48.68± 14.07 *#	40.82± 5.26 #
AST(U/L)	28.24± 4.84	74.10± 3.00 *	46.32± 6.70 *#	54.78± 3.60 *#	37.08± 6.52 #@

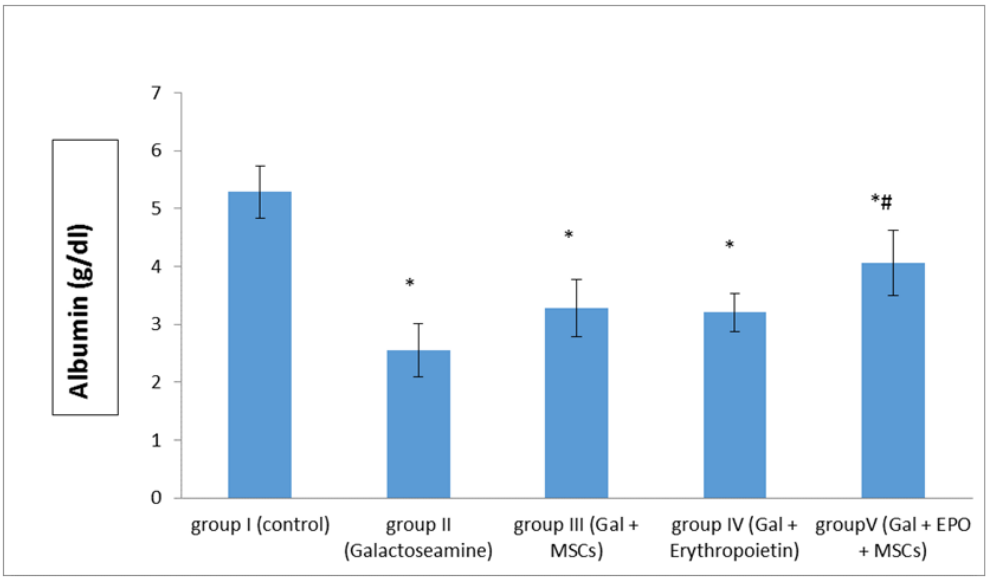


Fig. 1: Comparison between the levels of serum albumin in the different studied groups. **: statistically significant when compared to the corresponding value in the group (I) (P<0.05), #: statistically significant in comparison with the corresponding value in the group (II) (P<0.05).*

Mean Levels of Serum Albumin Show:

- Significant decrease in all groups versus the Control group.
- Significant decrease in Group II versus Control group and Group V.
- Significant decrease in Group III and Group IV versus Control group.
- Group V; Significant decrease compared to the Control group & significant increase compared to Group II (Table 1 & Fig. 1).

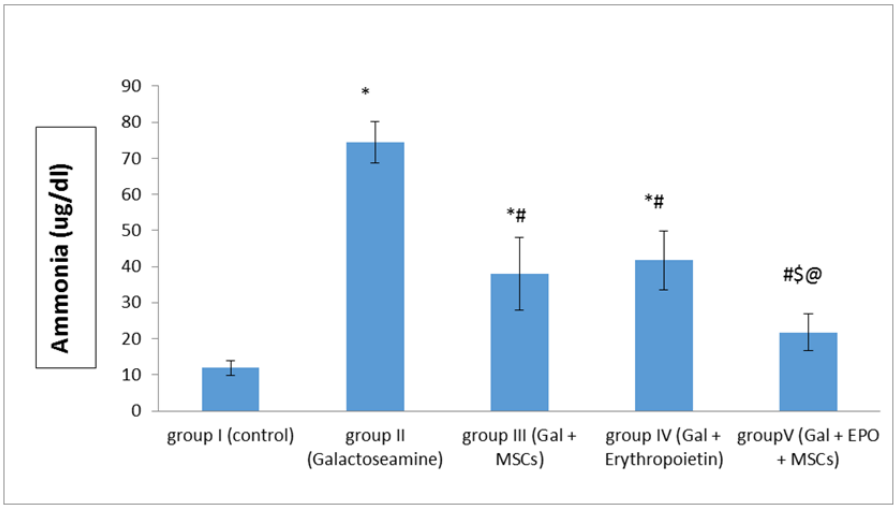


Fig. 2: Comparison between the levels of serum ammonia in the different studied groups.
**: statistically significant when compared to the corresponding value in group (I) (P<0.05), #: statistically significant compared to the corresponding value in group (II) (P<0.05), $: statistically significant compared to the corresponding value in group (III) (P<0.05), @: statistically significant compared to the corresponding value in group (IV) (P<0.05).*

Mean Levels of Serum Ammonia Show:
- Significant increase in Groups II, III and IV versus Control group.
- Significant increase in Group II versus other groups.
- Group III; Significant increase compared to Control group and Group V & The significant decrease compared to Group II.
- Group IV; Significant increase compared to Control group and Group V & The significant decrease compared to Group II.
- Group V; Significant decrease compared to Group II, Group III and Group IV (Table - 1& Fig. 2).

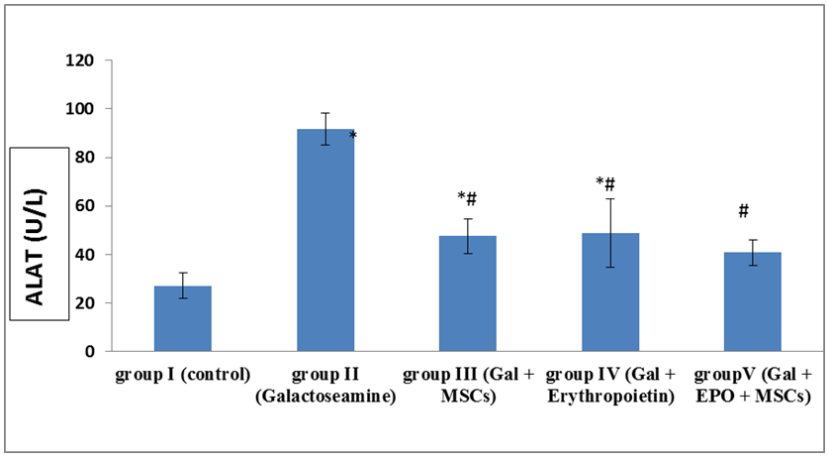


Fig. 3: Comparison between the levels of serum ALAT of different studied groups.
**: statistically significant compared to the corresponding value in the group (I) (P<0.05), #: statistically significant compared to the corresponding value in the group (II) (P<0.05).*

Mean Levels of Serum ALAT Activity Show:
- Significant increase in Groups II, III and IV versus Control group.
- Significant increase in Group II versus other groups.
- Group III and IV; Significant increase compared to Control group & significant decrease compared to Group II.
- Significant decrease in Group V compared to Group II (Table 1 & Fig. 3)

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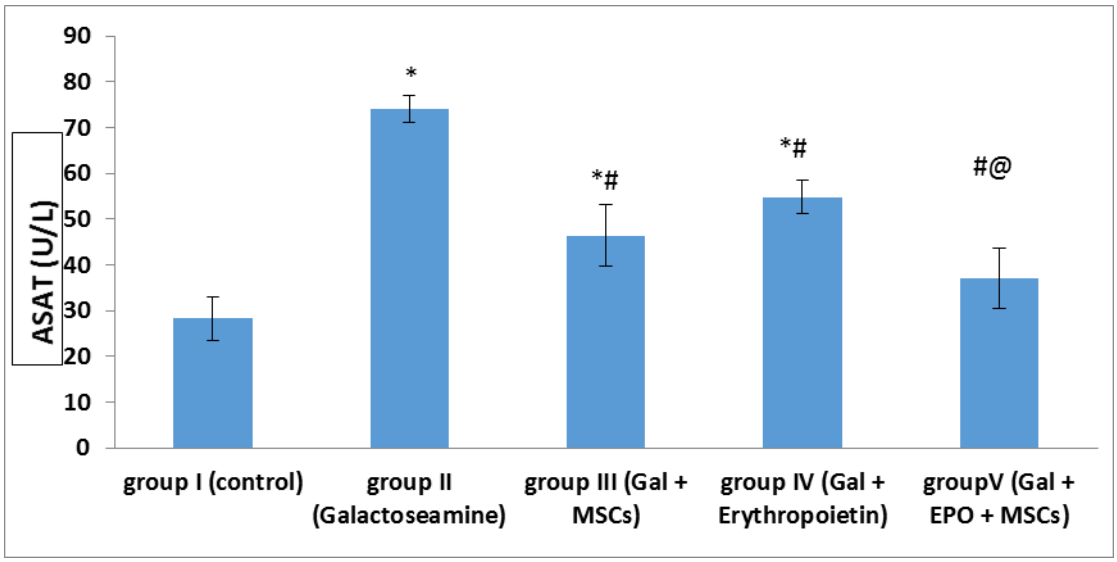
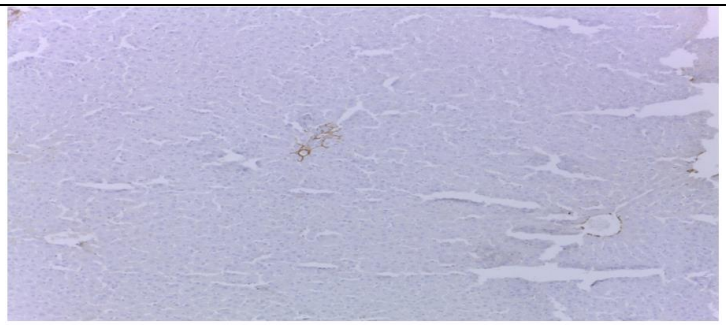


Fig. 4: Comparison between the levels of serum AST in the different studied groups.
**: statistically significant compared to the corresponding value in the group (I) (P<0.05), #: statistically significant compared to the corresponding value in the group (II) (P<0.05), @: statistically significant compared to the corresponding value in the group (IV) (P<0.05).*

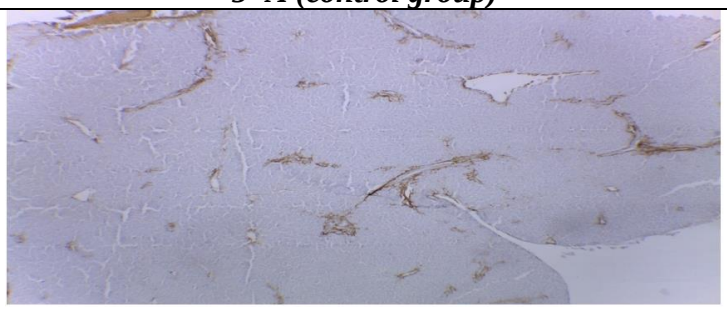
Mean Levels of Serum ASAT Activity Show:
- Significant increase in Groups II, III and IV versus Control group.
- Significant increase in Group II versus other groups.
- Group III; Significant increase compared to Control group & significant decrease compared to Group II.
- Group IV had a significant increase compared to the Control group and Group V & had a significant decrease compared to Group II.
- Significant decrease in Group V compared to Group II and Group IV (Table 1& Fig. 4).

Immunohistochemical Study of COX-2 (Fig. 5):

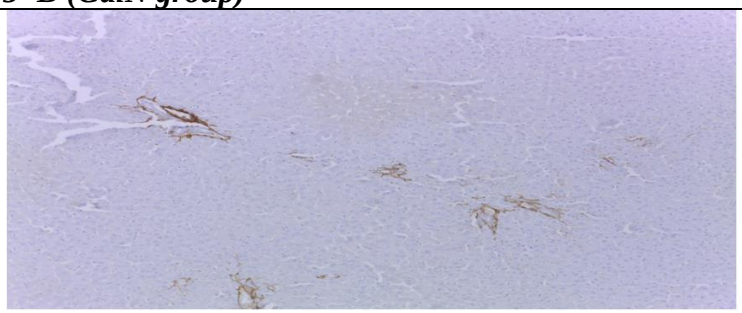
COX-2 (brown pigment) immunohistochemical study shows normal COX-2 distribution in the control group's liver tissue (Fig. 5- A). Increased COX-2 distribution in liver tissue appears in the GalN-injured group (Fig. 5- B). The protective effect of each MSCs and EPO appears with decreased COX-2 levels in pre-injured animals with GalN (Fig. 5- C & Fig. 5- D). The highly protective effect of both MSCs and EPO with additive effect appears with MSCs/EPO group, which appears near normal (Fig. 5- E).



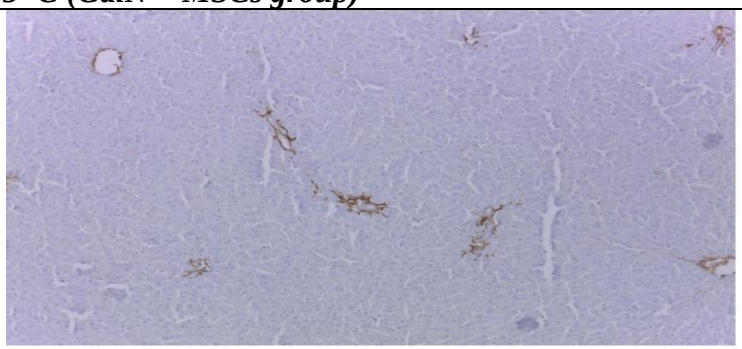
5- A (control group)



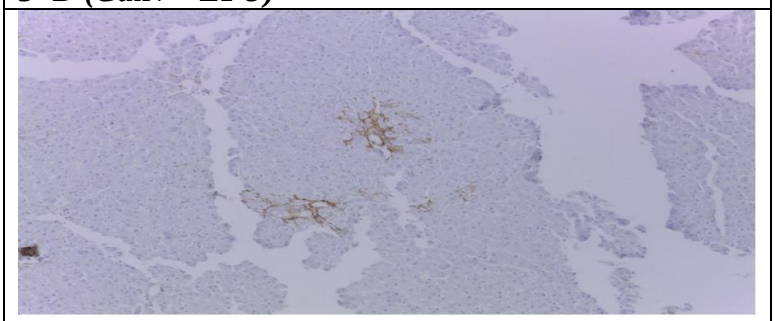
5- B (GalN group)



5- C (GalN + MSCs group)



5- D (GalN + EPO)



5- E (GalN + MSCs + EPO)

Fig. 5: Immunohistochemical study of liver tissue in the different groups.

DISCUSSION

MSCs are considered an effective seed cell for liver fibrosis treatment due to their properties; firstly, they exhibit minimal immunogenicity and immunosuppression and exhibit a wide immunomodulatory function range impacting the innate and adaptive immunities (*Li* L., Chen W., *et al.,* 2016), and benefit autologous and allogeneic transplants. Secondly, the intrinsic and extrinsic MSCs have Erythropoietin (EPO). It is a glycoprotein hormone that, in addition to its involvement in erythropoiesis, has been found as producing Epo and/or expressing the Epo-R in different cells like the endothelial, smooth muscle, and the central nervous system cells (Ogunshola and Bogdanova, 2013).

Different hepatoprotective agents have been identified for liver damage in animal models. Erythropoietin (EPO) is one of these agents. It is produced by the kidney. It increases the erythroid progenitor cell proliferation and differentiation. The recombinant human erythropoietin (rhEPO) is commonly utilized to treat renal diseases, iron deficiency, and chronic anemia. The EPO and its equivalents have been shown to impact the apoptosis and inflammation interaction in the kidney, liver, and myocardium (Ben-Ari *et al.,* 2011). EPO stimulates the endothelial progenitor cells' proliferation and protraction. It has a pivotal role in the marrow-derived MSCs' proliferation and differentiation (Liu *et al.,* 2011-b).

MSCs represent a hopeful gate and a new protective strategy not only in regenerative medicine but also in acute diseases like ALF. In comparison with the GalN-injected group, the MSCs-treated group had a drop in the ALT and AST levels and an increase in the albumin and ammonia levels. Prasajak and Leeanansaksiri (2014) mentioned some MSCs' unique mechanisms like the engraftment property, paracrine secretion activity, and trans differentiation capacity. The engrafted cells may develop into hepatocyte-like cells like the human albumin or α-fetoprotein, which may enhance the liver function by reducing the ALT and AST levels (Yan *et al.,* 2009).

This research aimed at studying the EPO protective effect in GalN-induced liver injury. We figured out that, like the MSCs, EPO had the same impact over the ALT, AST, albumin, and ammonia. This is matched with a recent study that showed that EPO significantly reduced the ALT and AST levels compared to the GalN/LPS group activities (Yang *et al.,* 2014).

Whether through trans differentiation capacity of MSCs, engraftment property, or cell fusion (MSCs fuse with another cell to create syncytium, a multinuclear cell) (*Liang et al., 2014),* MSCs can correct GalN induced liver toxicity. While several findings demonstrate that recombinant human EPO may enhance liver function and stimulate liver regeneration due to its anti-apoptotic, mitogenic, and tissue-protective multifunctional cytokine properties (Peng *et al.*, 2014).

In the present work, MSCs and EPO also expressed their role in acute liver injury at the level of COX-2. An immunohistochemical study revealed decreased quantity of expressed COX-2 with MSCs and/or EPO treatment compared to the GalN-injured group.

Apoptosis is programmed cell death known for nuclear fragmentation and cytoplasmic condensation. There are two types of apoptosis signaling pathways, receptor-mediated and mitochondrial-dependent apoptosis, which happen as a result of specific liver injuries and drug administration (Raj *et al.*, 2011).

Cyclooxygenase-2 (COX-2) is a vital inflammatory marker which is an inducible enzyme that stimulates the first step of the synthesis of prostanoids. Goradel, (2019).

Previous studies proved that the COX-2 genetic overexpression accelerates endotoxin-induced inflammation and subsequent liver damage. (Han *et al.*, 2008).

The present work showed an increased distribution of COX-2 in GalN-injured group versus the control group, this agrees with the (Kim *et al.*, 2015) results *who* stated that the elevated mRNA and hepatic COX-2 protein expression in GalN-treated animals. Highly protective effect of both MSCs and EPO with an additive effect which appears near normal. This is consistent with the (Abdel-Salam *et al.*, 2016) findings.

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