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The Potential Role of Bone Marrow-Mesenchymal Stem Cells on Sodium Nitrite - Hypoxia Model in Liver of Male Rats.

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ABSTRACT

A limited oxygen supply leads to hepato-cellular injure and cell death. Sodium nitrite induced hypoxia and hepatic damage**.** The current work aims to examine the potential role of stem cells on biochemical markers, histopathological and ultrastructure alterations of hypoxia caused by sodium nitrite ($NaNO_2$) toxicity in the liver of male rats. Adult male rats were divided into 6 groups. Group 1 (G1) was the control group. Group 2 (G2) received daily $NaNO_2$ (35 mg/kg bwt/ day) via subcutaneous injection for 3 weeks. Group 3 (G3) received $NaNO_2$ for 2 weeks and were then injected once with $2x10^6$ mesenchymal stem cells (MSCs) intravenously and sacrificed 4 weeks later. Group 4 (G4) treated as group 3 followed by daily $NaNO_2$ injection for 1 week; rats in G4 were sacrificed 4 weeks from MSCs treatment. Group 5 (G5) rats were treated with $NaNO_2$ for 2 weeks and then left to recover for 4 weeks. Finally, Group 6 (G6) rats were treated with $NaNO_2$ for 3 weeks and left to recover for 3 weeks, after which point they were sacrificed. The results showed that $NaNO_2$ caused oxidative damage and histopathological alterations in the rat liver, as well as increased the levels of liver oxidative stress markers in comparison with the control group. Nevertheless, the administration of stem cells reduced the danger actions of sodium nitrite by enhancing biochemical marker concentration. The definite of improvement of histo-pathological lesions is indicted in response to MSCs therapy.

Keywords: MSCs; Liver; $NaNO_2$ toxicity; Oxidative stress; Liver histology.

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INTRODUCTION

Hypoxia has been shown to have a role in the pathogenesis of several forms of liver disease [1]. Sodium nitrite under certain conditions can break down into a different substance known to cause Alzheimer's disease, cancer and fatty liver disease. Reduced ATP levels in the liver are the main factor that contributes to liver cell death [2]. The adverse cellular effect related to hypoxia exposures are mediated through generation of ROS and is underlying reason for many clinical disorders as well as hepatic cell death [3].

A limited oxygen supply leads to hepato-cellular injure and death. Those severe morphological changes (dilated congested blood vessels, necrosis, collection of inflammatory cells, condensed heterochromatic with irregular outlines nuclei and mitochondrial degeneration in the hypoxic liver [4]. The rats exposed to chronic systemic hypoxia showed liver tissue response by analyzing the oxidative stress [5]. Moreover, sodium nitrite-induced hypoxia results in severe necrotic change in the rat hepatocytes of the liver particularly in periportal region [6]. In the same trend, the decrease in ATP level in the liver of hypoxic rats, accompanied by an increase in AMP and ADP concentrations was observed [7]. The Cod liver oil ameliorates sodium nitrite-induced hepatic damage through multiple mechanisms counting blocking NaNO2-induced increasing of inflammatory cytokines, apoptosis markers, and fibrosis mediators [8]. The stem cells produce all multi-cellular tissues in the body through proliferation and differentiation in tightly controlled. Stem cells are currently used in clinical applications to augment the healing of orthopedic tissue defects [9]. Their applicability to multiple other therapeutic situations has also been investigated [10]. In contrast, liver regeneration after loss of hepatic tissue does not depend on these kinds of cells, but on the proliferation of the existing mature hepatocytes, In addition, other cells such as endothelial cells, Kupffer cells, may also contribute to regeneration of the lost hepatic tissue [11].

Previous studies have effort to exploit the potential of mesenchymal stem cells (MSCs) to differentiate and so replace harm resident cells, for example endothelial cells, cardiomyocytes, smooth muscle cells or hepatocytes, and thereby enhance tissue regeneration in various organs such as the kidney, liver, Many preclinical and clinical researches have provided growing proof of the efficacy of MSC-based treatments [12,13]. The intravenous infusion of MSC populations extended in vitro, some MSCs consequently do home to injured tissue, for example infracted myocardium and fibrotic liver [14]. MSCs, also known as multipotent mesenchymal stromal cells, are self-renewing cells that can be found in almost all postnatal organs and tissues, including liver [15]. During the past decade, great progress has been made in the field of MSC-dependent liver regeneration and immunomodulation. Because of their potential for differentiation into hepatocytes as well as their immunomodulatory characteristics, MSCs are considered as promising therapeutic agents for the therapy of acute liver failure and cirrhosis. So, the present study aims to evaluate the potential therapeutic role of MSCs on the hypoxic liver of rats treated with sodium nitrite.

MATERIAL AND METHODS

Experimental animals

In the present study, 96 adult male albino rats (weighing 150-180 g) were used. Rats were obtained from Ain Shams Hospital Animal House. Rats were left for two weeks for acclimatization before starting the experiments. Animal procedures and experimental protocols were approved by Ain Shams University authorities; they were in accordance with the Egyptian animal protection rules and consistent with the guidelines of the European Communities (EC) (1986).

Sodium nitrite ($NaNO_2$) administration

$NaNO_2$ (7632-00-0) was obtained from Alahram Company (Al-Sadat city, Egypt) and dissolved in distilled water at 27°C. It was administered subcutaneously to rats at a dose of 35 mg/kg bwt/day [16].

Isolation, propagation, identification and labeling of MSCs derived from bone marrow of rats

Bone marrow was removed from the tibiae and femurs of male rats, aged 6 weeks old and prepared as described clearly [17]. Cells were intravenously injected once into the rat tail vein at a dose of $2*10^6$ cells, according to previously published [18].

Experimental Design

Rats were randomly split into six main groups with 16 rats each. Group 1 (G1) received distilled water and served as the control. Group 2 (G2) received daily subcutaneous (s.c.) $NaNO_2$ injections at a dose of 35 mg/kg bwt/ day for 3 weeks. Group 3 (G3) received $NaNO_2$ for 2 weeks and were then injected once with MSCs ($2*10^6$ cells) intravenously; rat were sacrificed 4 weeks after MSC injection. Group 4 (G4) were treated with $NaNO_2$ for 3 weeks and treated also with MSCs after 2 weeks of $NaNO_2$ injection; rats were sacrificed 4 weeks from MSC injection. Group 5 (G5) were treated with $NaNO_2$ for 2 weeks and then left to recover for 4 weeks and then sacrificed. Lastly, Group 6 (G6) received $NaNO_2$ for 3 weeks then left to recover for 3 weeks and then sacrificed.

Biochemical Studies

Liver samples were prepared by taking a weighted part of the tissue and homogenizing in the recommended PBS saline. Biochemical analyses were conducted, which included colorimetric determination of NO [19] using modified Griss reagent, measurement of malondialdehyde in the tissue [20]**,** percentage of DNA fragmentation (as determined by quantitative analysis using diphenylamine assay, according to the method described by [21] and catalase activity was determined as described by [22]**.** Moreover, total antioxidant activity was verified according to [23].

Histopathological and Ultrastructure Investigation

In the present study, liver specimens were carefully dissected and fixed in Bouin's solution for routine histological analysis, and implanted in paraffin wax at 60oC. Serial transverse sections were then cut at 5-6 microns in thickness using Cambridge Rocking Microtome and affixed to slides. For general histological examination, sections were stained with Hematoxylin and Eosin [24]. Liver tissues were prepared for TEM using the procedures described according to [25]. Stained grids were examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Statistical Analysis

Reported data represented the mean ± SE for 8 animals per group. For statistical analysis, one-way analysis of variance (ANOVA) and post-HOC test ("least significant difference (LSD) analysis) were completed using statistical package for social science (SPSS) for Windows software (version 17). Statistical significance was set at $p<0.05$.

RESULTS

Biochemical investigation

The results of the biochemical parameters are shown in Table 1. Oxidative stress parameters showed a significant increase in all treated groups with regards to the following: the value of liver nitric oxide (NO) contents, malondialdehyde (MDA) contents, and DNA fragmentation percentage (DNA F %). On the other hand, there was a significant reduction in catalase activity (CAT) and total antioxidant activity (TAA) of G2 group as compared to the G1 control group. Moreover, there was a significant reduction in the recovery groups and the MSC treated groups with respect to liver NO contents, MDA contents, and DNA F%, when compared with those rats in G2 group. Conversely, liver catalase activities (CAT) and total antioxidant activities (TAA) were ignificantly increased in stem cell groups and recovery groups, compared to G2 rats.

Table (1): Total nitric oxide (NO) contents in the liver (mMol/g), malondialdehyde (MDA) contents (uMol/g), DNA fragmentation percentage (DNA F %), catalase activities (CAT) (u/g/sec), and total antioxidant activities (TAA) (mMol/g/min) are shown for the various treated groups.

Parameters Groups	NO	MDA	DNA F%	CAT	TAA
G1	46.93±0.681	37.68± 0.743	23.55±0.547	0.47±0.013	213.3±1.26

G2	101.07±0.801[a]	124.76±1.908[a]	46.86±0.181[a]	0.14±0.004[a]	32.5±0.75[a]
G3	56.97±0.768[ab]	44.71±0.999[ab]	31.49±0.911[ab]	0.36±0.011[ab]	158.2±2.27[ab]
G4	75.94±0.416[abc]	51.02±1.357[abc]	43.93±0.407[abc]	0.31±0.010[abc]	136.6±3.96[abc]
G5	76.18±0.687[abc]	62.37±0.836[abcd]	44.10±0.296[abc]	0.29±0.003[abcd]	118.1±5.15[abd]
G6	85.32±2.960[abcde]	94.71±3.857[abcde]	44.90±0.084[abc]	0.28±0.010[abcd]	96.6±2.50[abcde]

Values are means of 8 rats ± SE at $p<0.05$. 'a' represents significant change from control group (G1), 'b' represents significant change from hypoxic (G2) group, 'c' represents significant change from G3 group, 'd' represents significant change from G4 group, and 'e' represents significant change from group treated with sodium nitrite for 2 weeks then left for recovery period (G5).

Histopathological and Ultrastructure investigation

The examined liver in normal rats is covered by a capsule of connective tissue. The parenchyma is formed of a more or less regular cords arranged radials from the hepatic vein. Hepatic cords are generally one cell layer thick (**Fig.1)** and consisted of large polyhedral hepatic cells. The radically arranged plates of hepatocytes each of them having double prominent nuclei with an eosinophilic finely granular cytoplasm, and well developed kupfer cells scattered in blood sinusoids (**Fig. 2).** Fine reticular tissue network penetrates the parenchyma bounding sinusoidal blood vessels.

Light microscopic examination revealed that severe alteration of rat liver of Sod. Nitrate administered rats, after daily injection with $NaNO_2$ for 3 week G2 when compared to liver of control rats, a branches of the portal vein, hepatic artery and bile duct are representing in the portal triad lies the corners of the lobule light microscopic examination of rat liver revealed that sodium nitrite induced severe alteration when compared with control liver. In the liver of a rat treated with nitrate at dose (35mg/kg b.wt/day) for 3 weeks daily showing hypertrophied hydropic degenerated hepatocytes **(Fig. 3).** In addition, the section of liver in hypoxic rat showed cytoplasmic vacuolated hepatocytes and hyaline material in blood sinusoids **(Fig. 4).** Besides that the **figure (5)** illustrated hydropic degenerative cells apparent interstitial oedema and loss of liver architectures. In addition, giant cells with homogenous cytoplasm and showing lymphocytes infiltration within narrowing blood sinusoids **(Fig. 6).** On the other hand, the portal ductile proliferative reaction accompanied with focal necrotic areas**.** Also, atrophic and degenerative changes were illustrated in the hepatocytes around central vein, which were dilated and contained lysed red blood cells (**Fig. 7)**. A massive number of apoptotic bodies were observed with activated Kupffer cells in sinusoidal spaces, rupture of endothelial lining of central vein **(Fig. 8).** An improvement of histo-pathological lesions is indicted in response to MSCs therapy in the G3 and G4 showed repairing of liver cells; some areas appeared nearly normal persistent focal degenerative vacuoles within dilated blood sinusoids all over the liver tissue (**Fig. 9).** In the portal area, showing well developed bile ductile**.** In contrast, the liver section in figure **(10)** exhibited Fibrous bands around the central vein congested with foam cells and eventually with regenerative centri-lobular areas, accompanied significant interstitial lymphocytes inflammation**.** The improvement of histopathological lesions is indicted in response to MSCs therapy.

In liver rats administrated (35 mg/kg b.w) of $NaNO_2$ for 2 weeks + MSCs showed repair of liver cells; some areas appeared nearly normal persistent focal degenerative vacuoles within dilated blood sinusoids all over the liver tissue **(Fig. 11).** Organizational arrangement and the integrity of the hepatic lobule remained intact. The portal tracts were invaded with inflammatory cells consequence and nearly normal structure of the bile duct as showing in figure (**12**). The liver tissue of G5 and G6 exhibited repairing of liver cells partially similar to normal hepatocytes, accompanied by rupture in the central vein endothelial lining and little activated kupffer cells around central vein **(Fig. 13).** Also, pyknotic hepatocytes nuclei and slight lymphocyte infiltration in widened blood sinusoids**.** The liver section of rats exposed to hypoxia for 3 weeks then left to make recovery showed ruptured wall of central vein with hyaline material within endothelial layer **(Fig. 14)** with numerous lymphocyte infiltrations.

Ultra structural investigations, of the liver cells of control rats, was mainly represented by a large number of mitochondria have normal size and arranged cristae in association with rough endoplasmic reticulum (R.E.R) and well developed Golgi apparatus, Large round nucleus contained chromatin, with well-defined nuclear envelope **(Figs. 15)**. On the other side, electron microscope findings of the liver tissue of G2 represented in the **figure (16**) illustrate marked change of hepatocytes represented by plasma membrane

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blebs and lysosome body (short arrow). Other lesions were noticed as disintegration of most cytoplasm organelles, the nuclear membrane destroyed (long arrow), lyses mitochondria (short arrow) and ill-defined endoplasmic reticulum was showed in **figure (17)**. A febrile capsule with nucleoli segregation (long arrow) in addition of lyses the destroyed swollen mitochondria with empty degenerative matrix as showing in **figure (18)**. Also, there are dilated (SER) (heads), degenerated hepatocyte nucleuses with faintly appearance of destroyed mitochondria. Note, condensed nucleus, disintegration of its envelope, variable size of lipid droplet and Lysosome. Irregular nucleus and vacuole, where as in the liver tissue followed by stem cell treatment**,** electron micrograph investigation of G3 revealed marked improvement in mitochondria self-division and RER lamella (cisternae) (long arrow) can be observed in the **(Fig. 19)**. **In the** structures nearly to normal but in the other liver tissue of rat group G4 in **figure (20)** showing mild improvement of hepatocytes nuclear membrane with well defined as nucleoli (long arrow), rupturing of rough endoplasmic reticulum (SER) cisternae (short arrow).

After a period of recovery from hypoxia, the hepatocyte for rats of G5 showed numerous chromatin granules at periphery of condensed chromatin (long arrows), swelling of internal compartment with fragmentation of cristae, and little improved mitochondria **(fig 21).** A normal nuclear membrane with well-defined nucleoli (long arrow) and vacuolated SER cristae (short arrow) obviously clear in **figure (22)** for liver tissue after recovery period of a hypoxic rats administered sodium nitrite for 3 weeks (G6 group).

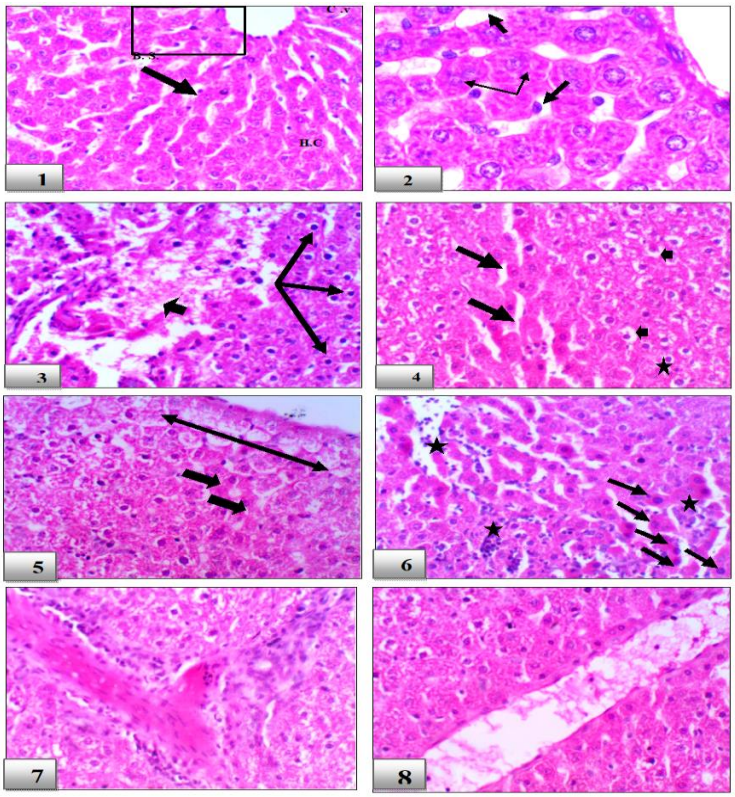

Figure (1) Normal liver section (Cont group) illustrating (C.V.) central vein, (H.C.) hepatic cord, (B,S) blood sinusoid (arrow) .The hepatic cords are generally one cell layer thick and the cytoplasm of the cells is homogeneous in normal liver (H&E X 200)
Figure (2) Magnification of previous part showing normal liver section large polyhedral hepatic cells having two nucleus (thin arrow), kupfer cells (thick arrows) (H&E X 1000)
Figure (3) liver section from Hypoxic rats (Hpx group) showing hypertrophied hydropic degenerated hepatocytes (tri-arrows), lymphocytes infiltration and necrotic portal zone (short arrow) (H&E X 400)
Figure (4) liver section from Hypoxic rats (Hpx group) showing cytoplasmic vacuolation hepatocytes cytoplasm (arrow heads) hydropic degeneration (star) hyaline material in blood sinusoids (arrows) (H&E X 400)
Figure (5) Liver section from Hypoxic rats (Hpx group) showing hydropic degenerative cells (double head), apparent interstitial edema, (thick arrows), loss of liver architectures and narrowing blood sinusoids (H&E X 400)
Figure (6) Liver section from Hypoxic rats (Hpx group) showing hyper chromatic giant cells with homogenous cytoplasm (short arrows), interstitial lymphocytes infiltration within sinusoids (stars) (H&E X 400)
Figure (7) Liver section from Hypoxic rats (Hpx group) manifesting the portal ductile proliferative reaction accompanied with hyaline material, focal necrotic areas (H&E X 400)
Figure (8) Liver section from Hypoxic rats (Hpx group) showing massive number of apoptotic hepatocytes, massively dilated portal interstitial space filled with hydropic vacuoles (H&E X 400)

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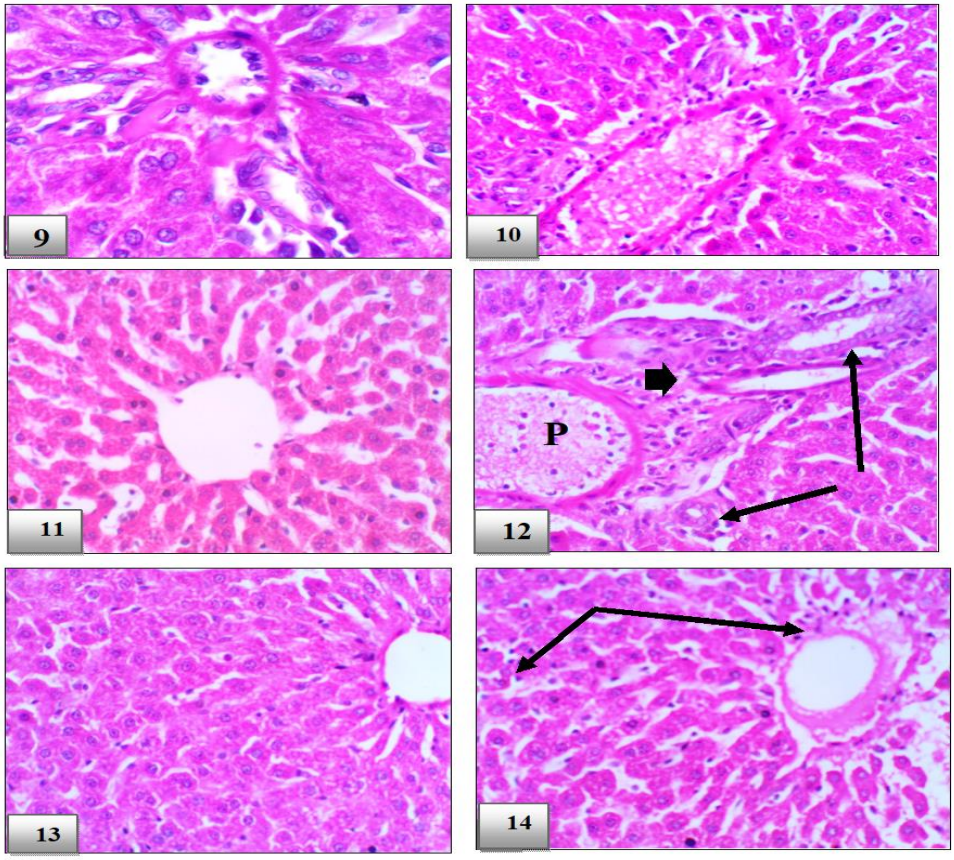

Figure (9) Magnification of portal area, showing well developed bile ductile (H&E X 1000)
Figure (10) Liver section from stem cell treatment rats (N-2WS group) showing Fibrous bands around the central vein congested with foam cells and eventually with regenerative centrilobular areas, accompanied significant interstitial lymphocytes inflammation (H&E X 400)
Figure (11) Liver section from stem cell treatment rats (N-3WS group) showing repairing of liver cells; some areas appeared nearly normal persistent focal degenerative vacuoles within dilated blood sinusoids all over the liver tissue (H&E X 400)
Figure (12) Liver section from stem cell treatment rats (N-3WS group) illustrates portal ductile proliferative reaction ( ) with inflammation, fibrous band around wide hyperemic portal vein (PV) and nearly normal structure of the bile duct ( ) (H&E X 400)
Figure (13) Liver section from rats left to recovery (N-2WR group) showing repairing of liver cells near to normal hepatocytes , the central vein endothelial lining appear normal, little activated kupffer cells around central vein (H&E X 400)
Figure (14) Liver section from rats left to recovery (N-3WR group) showing ruptured wall of central vein with hyaline material within endothelial lining of the central vein(star), and few lymphocyte infiltrations (2 arrows) (H&E X 400)

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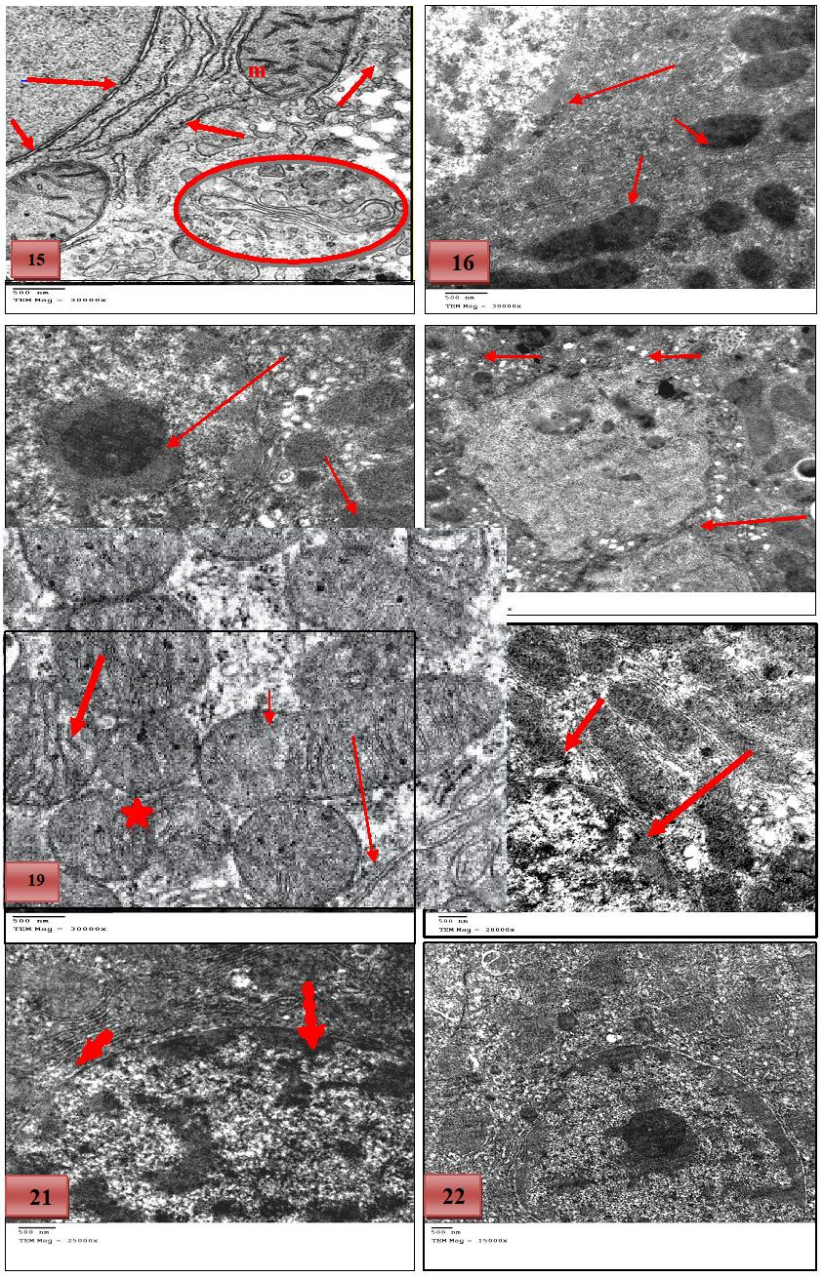

Figure (15) Electron micrograph of a hepatocyte of control rat showing normal sized mitochondria (m) large round nucleus (n) normal rough endoplasmic reticulum .(TEM Mag 30000 X)

Figure (16) Electron micrograph liver section from Hypoxic (Hpx group) rats showing the nuclear membrane destroyed (long arrow) lysed mitochondria (short arrow) and ill-defined endoplasmic reticulum (TEM Mag 30000 X)

Figure (17) Hypoxic electron micrograph liver section from Hypoxic (Hpx group) rats illustrating fibrillar capsule with nucleolar segregation (long arrow) and multigranular surrounding the lyses mitochondria which destroyed with degenerative matrix (short arrow) (TEM Mag 20000 X)

Figure (18) Hypoxic electro micrograph liver (Hpx group) section showing dilated SER due to swelling cells (heads) degenerated hepatocyte nucleus, with faint appearance of destroyed mitochondria (TEM Mag 15000 X)

Figure(19) Electron micrograph of a hypoxic hepatocyte for (N-2WS group) followed by stem cell treatment showing marked improvement in mitochondria and RER lamella (long arrow) with mitochondria self-division ( ) (TEM Mag 30000 X)

Figure (20) Electron micrograph of a hypoxic hepatocyte for (N-3WS group) followed by stem cell treatment Note numerous per chromatin granules at periphery of condensed chromatin (long arrows) swelling of internal compartment with fragmentation of cristae. little improvement mitochondria (TEM Mag 20000 X)

Figure (21) Electron micrograph of a hypoxic hepatocyte for (N-2WR) followed by recovery time, showing, nearly to normal nuclear membrane with well distributed chromatin material (long arrow), SER cristae (short arrow). (TEM Mag 25000 X)

Figure (22) Electron micrograph of a hypoxic hepatocyte for (N-3WR) followed by recovery time, showing, normal nuclear membrane with well-defined nucleoli (long arrow), vacuoled SER cristae (short arrow). (TEM Mag 15000 X)

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DISCUSSION

The liver is the most sensitive organ to pre-oxidative damage because it is rich in oxidizable substances. The more severe the liver damages the higher the release of the liver enzymes [26]. The effect of sodium nitrite administrated via multiple mechanisms including: (1) a reduction in sodium nitrite-induced oxidative stress, as indicated by reduced hepatic MDA levels and restored activity of hepatic GSHR and GSH-Px; (2) a blocking of sodium nitrite-induced increases in hepatic proinflammatory cytokines such as TNF and IL-1; (3) a reduction of sodium nitrite induced increases in acute inflammation markers such as CRP; (4) a blocking of sodium nitrite-induced increases in hepatic fibrosis markers such as MCP-1 and TGF- 1; (5) inhibition of sodium nitrite-induced deactivation of mitochondrial function as indicated by restoration of cytochrome C oxidase activity; and (6) a reduction in sodium nitrite-induced activation of hepatic caspase-3 and of the increases in the percentage of DNA fragmentation [8].

In present study the physiological effect of $NaNO_2$ on liver of rat showed an increase in the levels of NO as compared to the control one. These results can be explained by the interaction of NO with some oxygen free radicals such as superoxide yields peroxynitrite, which may further interacts with tyrosine residues in biological molecules forming nitrotyrosine [27]. In agreement with the present results, peroxynitrite was also suggested to be parted to form NO_2 and NO_3, which might involve in DNA damage [28]. The apparently conflicting theories of NO_2 - reduction and S-nitrosothiols acting as non-NOS-dependent sources of NO, and as regulators of local blood flow under both physiologic and hypoxic/ischemic conditions, may be reconciled [28,29]. Under physiologic conditions, NO_2 - is not directly reduced to NO but rather modulates many signaling pathways, including soluble guanylate cyclase activation. It also induces post-translational modifications normally associated with NO, such as the formation of nitroso- and nitrosyl species [29]. NO_2 - may therefore exert its signaling functions directly, without the need for intermediary form action of free NO. Hypoxia markedly potentiates tissue NO production from NO_2 - in a dose-dependent manner this occurs particularly in liver [30]. In the same trend, increased expressions of INOS and also nitrotyrosine were shown to take place in both liver and kidney tissues of mice received $NaNO_2$ [31].

The data obtained from the present study showed that hypoxia caused increase in liver MDA contents after administration of sodium nitrite, according to Özena and coworkers [31] found that MDA increased in $NaNO_2$ groups as compared to the control. The high oxidative stress indicator, MDA, could be attributed to the oxidative cytotoxicity of nitrite [32]**.** These results also proved by [33] which found that oxidative stress caused by exposure to intermittent hypoxia for 1 week resulted in a trend to an increase in MDA contents in liver tissue. Moreover**,** [34] reported that sodium nitrite and other food additives may react with amines of food in the stomach and produce nitrosamines and free radicals. Such products may increase lipid peroxidation (MDA), which can be harmful to different organs including liver [35,36]. Consistent with observations from these previous studies, [37] found significant increases in oxidative stress markers (MDA, hydrogen peroxide and superoxide anion) and significant decreases in antioxidant activity (SOD, catalase and reduced glutathione) in both serum and hepatic homogenates.

In present study the physiological effect of $NaNO_2$ (35m/kg Sc) on liver of rat showed an increase in the levels of DNA fragmentation percentage (DNA F %) as compared to the control one. Reactive oxygen species are believed to cause genetic oxidation and damage to DNA and other macromolecules [38]. Our result demonstrated that administration of sodium nitrite resulted in a significant increase in DNA fragmentation. The morphological features induced by high sodium nitrite completely reflected the classic apoptotic features. This was further confirmed by DNA fragmentation. In addition, lipid peroxidation induced by the free radicals of sodium nitrite combine with DNA to form adducts and accelerates DNA fragmentation [39]. However, they found that sodium nitrite exhibited a remarkable increase in hepatic DNA fragmentation percent.

The ROS production contributes to mitochondrial damage in a range of pathologies and is also important in redox signaling from the organelle to the rest of the cell [40-42] found that hepatocytes exposed to hypoxia displayed elevated expression of ROS, These results are in agreement with previous reports that the higher levels of ROS are a major damaging factor to hepatocytes. The centrilobular necrosis occurs in situations of important liver ischemia since the hepatocytes in this zone are more sensitive to hypoxia as a consequence of the type of characteristic circulation in the liver. Light microscopic examination revealed that severe alteration of rat liver of sodium nitrite [43].

The present study indicated an increase in the levels of MDA and NO in liver in hypoxic group compared to control one. Treatment with MSCs significantly restored the levels. CAT and TAA activity have significantly increased in MSCs treated groups whereas toxic group has shown a significant decrease in levels compare to the control group. These results could explain as follows: When tissue damage occurs, specific endocrine signals are released from the injury site that mobilizes MSCs from bone marrow to the location of damage [44]. Furthermore, small amounts of MSCs are retained in the blood circulation and have the ability to home to injury sites for tissue repair [45]. Their utility lies in the fact that MSCs may serve as primary scaffolds for new tissue and secrete protective humoral factors in certain diseases [46, 47]. MSCs are also able to migrate to sites of inflammation and tissue injury while modulating the immune response [48-50]. One theory of tissue repair holds that organ injury is "sensed" by stem cells that migrate to the site of damage and differentiate into organ-specific cells, promoting structural and functional repair [51, 52].

In the present study**,** the treatment with the MSCs led to a significant decrease in hypoxic groups in NO and MDA contents as compared with the control one. Also, a significant increase of CAT, TAA activities was detected. In support of the present results [53] reported that transplantation of MSCs can correct and reverse the imbalance between ROS and antioxidant defense in favor of antioxidant defense by restoring and augmenting its capacity as well as modulating lipid peroxidation. The present results are agreed with [54] who demonstrated the anti-oxidative role of the MSCs. The in vivo protection by MSCs induced oxidative damage may be because of its free radical scavenging potential. The specific responses of mesenchymal stem cells to oxidative stress may play a crucial role in regulation of tissue homeostasis as well as regeneration of organs after oxidative injury [55,56]. It could also be because of direct scavenging/neutralization of the free radical or induction of the endogenous antioxidant enzymes such as CAT and SOD.

Nitrite administered rats, after daily injection with (35 mg/kg b.wt/day, Sc) of $NaNO_2$ for 3 week (G2), illustrated that the hypoxic liver section showed necrosis, hypatocytes cytoplasm vacuolation and lymphocytes inflammation could explained by [57]. Who is found the failure of aerobic ATP formation by oxidative phosphorylation is the fundamental stress of anoxic and ischemic injury. The importance of ATP depletion in the events leading to necrotic cell death is demonstrated by the ability of glycolytic substrates to rescue hepatocytes and endothelial cells in sinusoidal space from lethal cell injury. Hydropic degeneration, hyaline material and lymphocytes inflammation was detected in agreed with the result of [8] who suggested that sodium nitrite-induced elevation of inflammatory cytokines, fibrosis mediators, and apoptosis markers. In this context, hypoxia acts not only as an aggravating factor of cell damage and inflammation, but also as an inhibitor of liver regeneration, a major stimulus of angiogenesis and fibrogenesis, and a promoter of liver carcinogenesis, Lack of oxygen also causes metabolic cell death; increased oxygen concentrations carry a risk for oxidative damage to proteins, lipids and nucleic acids, possibly initializing apoptosis or carcinogenesis [58]. Liver injury causes vascular disorganization and local tissue hypoxia starting early in disease course. In this context, hypoxia acts not only as an aggravating factor of cell damage and inflammation, but also as an inhibitor of liver regeneration, a major stimulus of angiogenesis and fibrogenesis, and a promoter of liver carcinogenesis **.**

In response to chronic hypoxia, the capacity of red blood cells to transport oxygen is up-regulated by the expression of genes involved in erythropoiesis, notably the erythropoietin (EPO) gene is increased by hypoxia, which is required for the formation of red blood cells. An increase of the number of the red blood cells enhances the delivery of oxygen to tissues [59] *WHICH* coming with our observations in the tissues liver especially in that groups of rats administrated $NaNO_2$ for 3 weeks, and that explain the central vein congested in liver tissue and congested blood capillary in the intertubular space in the liver tissue. Lack of oxygen causes metabolic cell death; increased oxygen concentrations carry a risk for oxidative damage to proteins, lipids and nucleic acids, possibly initializing apoptosis [60].

[61] Described that liver of sodium nitrite administered groups showing increased number of binucleated hepatocytes with hepato-cytomegally, marked sinusoidal dilatation associated with kupffer cell activation, bile duct hyperplasia with newly formed ductules, oval cell hyperplasia and portal mononuclear cell infiltration and sever hepatocellular necrosis and vacuolization. This is come together with our investigations that were detected also by [62]. [63] attributed the previous features to the more capable of responding to a major demand for protein synthesis, or may be a reactive cell response to liver injury. The observed vascular dilatation may represent an adaptive process as an attempt to overcome this oxygen deficiency which prolonged the causes of atrophic cell's formation. Atrophied liver cells probably later, as results of necrotic

patches. Also, the dilatation of the sinusoids may be attributed to hepatic congestion that results from a direct action of the treatment on the vessel wall or the back pressure in the portal space, this is in accordance with [64] who support this observations, liver damage was also recorded by [65] and the observed vascular dilatation may represent an adaptive process as an attempt to overcome this oxygen deficiency which, when prolonged, may be the cause of atrophic cells formation. Atrophied liver cells probably later, results in the presence of necrotic patches. Also, the dilatation of the sinusoids may be attributed to hepatic congestion that results from a direct action of the treatment on the vessel wall or the back pressure in the portal space [66].

Stem cells have the ability for prolonged self-renewal and differentiation into mature cells of various lineages, which makes them important cell sources for tissue engineering applications [67].

In this present study, the treatment with MSCs against hypoxic tissues effectively reduced the degenerative changes in liver cells. There were little hepatocyte cells manifested degenerative changes, thereby indicating MSCs prevented liver damage. This is consistent with the study of [12,13]. Similarly, MSCs has protective equipment against experimental liver fibrosis and repair of damaged hepatocytes [68,69]. In the same trend, the result of injected MSCs, also, showed therapeutic effects including repair of damaged hepatocytes, intracellular glycogen restoration, and resolution of fibrosis. Similarly, bone marrow derived MSCs showed protection against experimental liver fibrosis in rat's model. In the current results of our histological evaluation of rat liver tissues of G3 revealed that the stem cells give rise a markedly improvements in different tissue, stem cell therapy in each groups has significant ameliorative at the all examined tissues level. These results agree with work that studied the efficacy of mesenchymal stem cells of repair tissues toxicity in rats due to hypoxic effect [70]. Also *[71]* illustrated that after acute liver injury, hepatic stem cells take part in normal tissue repair and homeostasis quickly. The same view of the present study, which deals with the MSCs to differentiate and thus replace damaged resident cells, such as endothelial cells, hepatocytes, and thereby promote tissue regeneration in various organs such as liver. Many preclinical and clinical studies have provided growing evidence of the efficacy of MSC-based treatments**.** Moreover, [72] detected that the transplantation of hypoxia-preconditioned MSCs exerted better therapeutic effects induced pulmonary fibrotic and enhanced the survival rate of engrafted MSCs, partially due to the up regulation of hepatocyte growth factor. MSCs have the potential to be used for the treatment of liver diseases due to their regenerative potential and immunomodulatory properties. Furthermore, MSC therapy could provide minimally invasive procedures with relatively few complications, as compared to liver transplantation [73].

Furthermore adaptation to hypoxia was seen in the current results revealed that after recovery period of a hypoxic liver for rats groups (G5) and (G6) illustrating marked improvement of hepatocytes structures nearly to normal at the level of light microsccope investigations. On the other side, the rats (G6) exhibited repairing of liver cells partially similar to normal hepatocytes, accompanied by rupture in the central vein endothelial lining and little activated kupffer cells around central vein. This is in agreement with an adaptive role in hypoxia [74]. Also, pyknotic hepatocytes nuclei and slight lymphocyte infiltration in widened blood sinusoids was shown. The radiating cordlike arrangement of the hepatocytes was disturbed, except in the region around the central veins due to its nearest to the blood supply sours. This is restoration according to the recovery processes.

At the level of electron microscopic (E.M.) different ultra structural appearance of the rat liver treated with sodium nitrite for 3 weeks (G2)**,** there are plasma membrane blebs, the nuclear membrane destroyed, lyses mitochondria, and ill-defined endoplasmic reticulum was showed a fibrillar capsule with nucleolar segregation due to changes in protein composition occurred after $NaNO_2$ treatment, which agreements with [75].

In the present investigation, the E.M. Study of the hepatocytes of G2 treated rats exhibited cytoplasmic vacuolization and alterations of cell organelles, short lamellae of rough endoplasmic reticulum found in close association with swollen mitochondria which scattered freely within the cytoplasm, especially in the periportal region as previous mentioned. In addition, the hepatocyte response to toxic insult was also reflected by irregular shape of nuclei and nuclear condensation with cytoplasm vacuolation and mitochondrial swelling. Similar results were reported by [76], it was possible to determine the site and nature of the action of sodium nitrite on the plasma membrane of the enterocytes providing a possibility for producing lability of these membranes which is associated with changes in transport function. The structure of other membrane

lipids such as membranes of lysosomes or mitochondria might be changed. An interaction with the respiratory chain was found [77] which are coming with our findings.

Also**,** [78] found that increased membrane permeability to calcium, potassium and chloride ions which consequently disturbed the balance of ions in the liver cells which agreements with previous detected lesions in the hypoxic group such as vacuolation and mitochondrial swelling which confirm images of necrosis.

Mitochondria are an important source of ROS (reactive oxygen species) within most mammalian cells [79,80]. Other explanations of faint appearance of destroyed mitochondria with empty degenerative spaces in matrix was illustrated by [81] has been suggested that the mitochondrion acts as the site of hypoxia sensing, this is based on the fact that the mitochondria binds O_2 and represents the primary site of oxygen consumptions in the cell, in order that it is the first organelles can be affected. Also, there are dilated SER, degenerated hepatocyte nucleus with the lipid droplet dilated SER due to swelling cells degenerated hepatocyte nucleus.

At E.M. levels in the G3 and G4 groups showed that stem cells induced a significant improvement in the liver sections. There are nuclear membranes with well defined nucleoli, mild vacuoles smooth endoplasmic reticulum (SER) cristae. This is explained by [82] who detected that the Reoxygenation of hypoxic liver also promotes the formation of reactive oxygen species, including hydrogen peroxide (H_2O_2) and superoxide (O_2). After period of recovery time, a hypoxic liver hepatocyte for rats administered sodium nitrite manifested marked improvement of cell organelles nearly to healthy features.

CONCLUSION

Treatment with $NaNO_2$ induced hypoxia in the rat liver. $NaNO_2$ led to a significant decrease in CAT and TAA activities of liver tissues when compared with control rats. Moreover, the histological hypoxic liver sections showed necrosis, hypatocytes cytoplasm vacuolation and lymphocytes inflammation and revealed short lamellae of rough endoplasmic reticulum found in close association with swollen mitochondria which scattered freely within the cytoplasm, especially in the periportal region as previous mentioned. In addition, the hepatocyte response to toxic insult was also reflected by irregular shape of nuclei and nuclear condensation with cytoplasm vacuolation and mitochondrial swelling. Importantly, treatment of rats with MSCs improves toxicity associated with $NaNO_2$ induced hypoxia in the liver of the rats and effectively reduced the degenerative changes in hepatocytes. Overall, the data indicate that MSCs therapy can limit liver damage from hypoxia induced by $NaNO_2$.

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