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Research Article

MESENCHYMAL STEM CELLS VERSUS ANTIOXIDANT THERAPY IN ACUTE RENAL FAILURE INDUCED BY ISCHEMIA-REPERFUSION

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ABSTRACT

The study showed the effect of MSCs and antioxidants on amelioration of acute kidney injury induced by ischemia-reperfusion and the different possible underlying renoprotective mechanisms.
It was carried on 60 male Sprague-Dawley rats (body weight 170-220 g). Rats were divided into 6 groups as follow: Group 1 (Sham group), where 10 rats were subjected to right nephrectomy without exposure to left renal pedicle ischemia, (Group 2) Positive control group, where 10 rats were subjected to right nephrectomy and left renal ischemia, (Group 3) Treated 1 group, where 10 rats were subjected to right nephrectomy and left renal ischemia. This group was received DPPD (0.5 g / kg, i.p.) 24 hours before the induction of ARF, (Group 4) Treated 2 group, where 10 rats were subjected to right nephrectomy and left renal ischemia. This group was received DPPD (0.5 g / kg, i.p.) 24 hours after the induction of ARF, (Group 5) Treated 3 group, where 10 rats were subjected to right nephrectomy and left renal ischemia. This group was received DPPD (0.5 g / kg, i.p.) 48 hours after the induction of ARF, (Group 6) Treated 4 group, where 10 rats were subjected to right nephrectomy and left renal ischemia. This group was received MSCs, which were processed and cultured for 14 days, in a dose of (106) by IV infusion at the rat tail vein 24 hours after the induction of ARF. After 4 days of induction of the acute renal failure rats were sacrificed to obtain renal tissue, blood, urine specimens.
As aresult, I/R group showed marked thrombosis in glomerular capillaries with disrupted glomerular basement membrane, Leukocytic infiltration (glomerular necrosis), marked tubular necrosis, interstitial haemorhage and inflammation and normal blood vessels. Serum levels of B-N-Acetylglucosaminidase (NAG), urea and creatinine were significantly elevated. Lipid peroxidation showed marked elevation. Both MSCs and antioxidant ameliorated I/R-induced nephrotoxicity to a great extent and showed significant anti-renal injury.

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INTRODUCTION

AKI known as a sudden decrease in renal function, resulting in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products. Frequently, this situation is also accompanied by metabolic disturbances, such as metabolic acidosis and hyperkalaemia, changes in body fluid balance which will be harmful for many other organs. AKI was classified from severe (i.e. requiring dialysis) to slight increases in serum creatinine (S-creatinine) concentration (e.g. 44μmol/l x 2). The most common classification used is Risk, Injury, Failure, Loss and End-stage renal failure (RIFLE) criteria. It use S-creatinine and urine output to classify AKI into three severity categories (risk, injury and failure) and two clinical outcome categories (loss and end-stage renal disease) (Lameire *et al*, 2013).

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I/R injury is a common cause of AKI beside several causes that may lead to ischaemia in the kidneys. Such as anaesthesia, cardiacsurgery, haemorrhage and gastrointestinal fluid losses may all cause I/R-induced AKI. Initially, all these causes lead to inadequate amounts of effective intravascular blood volume and impairment of kidney blood flow. This causes decrease in O2 and nutrient supply to the kidneys and accumulation of metabolic waste products, which cause cell damage. Inflammation and oxidative stress may be resulting from the reperfusion process, as well as endothelial and vascular dysfunction (Bonventre *et al*, 2011).

Cellular respiration and metabolism continuously produce low levels of ROS, including superoxide (O_2^-). O_2^- acts as a precursor for more dangerous ROS, such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$). ROS tend O_2 tension and regulate signal transduction from membrane receptors in many physiological processes. In addition, Low concentrations of ROS control vascular tone through vascular smooth-muscle cell relaxation. Normally, Antioxidants protect the body itself against various types of oxidative damage caused by reactive oxygen species (ROS). For example, vascular superoxide dismutase (SOD) removes small concentrations of O_2^- (Lakshmi *et al*, 2009).

There is a relation between the nephrotoxicity of I/R and the ROS activity. Free radicals interact with thiol groups and macromolecules then make them inactive. Similarly, hydrogen peroxide is very reactive towards the cysteine residue of glutathione, which detoxifies hydrogen peroxide by a rapid binding mechanism. Many antioxidants have activity to either improve or prevent the nephrotoxicity (Jansen BA *et al*, 2002). Kidneys can be recovered from I/R injury that increase cell death. However, I/R-induced AKI can also lead to acceleration of CKD especially in humans with underlying CKD (Ishani *et al*, 2009).

The turnover rate of human proximal tubule cells increases after I/R-induced AKI when cell death is increased by necrosis and apoptosis. However, this repair process may also be maladaptive and I/R-induced AKI can lead to incomplete tubular repair, chronic tubulointerstitial inflammation, proliferation of fibroblasts and increased formation of extracellular matrix (ECM). For example, chronic hypoxia, resulting from loss of peritubular microvessels, and chronic activation of macrophages, may also contribute to the development of fibrosis after I/R injury (Duffield *et al*, 2010).

There are reports that antioxidants such as N N'-diphenyl-1, 4-phenylenediamine (DPPD) inhibit interstitial fibrosis induced by cisplatin (Sato S *et al*, 2002; Sato S *et al*, 2005). It prevents the increases in content of lipid peroxides and nephrotoxicity induced by cisplatin, where antioxidants are able to make trapping for free radicals (Sugihara K *et al*, 1987).

Many recent researches in the field of regenerative medicine have referred to the use of stem cells. Stem cells are undifferentiated cells (Blank cells) that have self-renewal properties. (Solter D *et al*, 1999). The bone marrow is the source of mesenchymal SC (MSCs) from which many tissues may be obtained. The ability of adult MSCs to "transdifferentiate" could revolutionize regenerative medicine (Rosenbaum *et al*, 2008).

Mesenchymal stem cells are of great interest because of their marvelous ability to enhance tissue engineering. MSCs are easily to be isolated and handled, also MSCs have great extent to be differentiated and specified. (Rosenbaum *et al*, 2008). MSCs can repair the injured kidney through variable mechanisms where stem cells are able to make Homing to the injured kidney and secretion of anti-inflammatory cytokines like dampen the inflammatory response also it differentiate into tubular epithelial cells (Zahran F *et al*, 2014).

This work aims to study the effect of mesenchymal stem cells (MSC) and N N'-diphenyl-1, 4-phenylenediamine (DPPD) as a line of treatment for acute renal failure induced by ischemia-reperfusion.

MATERIALS AND METHODS

The study was carried on 60 male Sprague-Dawley rats (body weight 200-250 g, 4-6 months old). Rats were bred and maintained in an air-conditioned animal house with specific pathogen free conditions, and were subjected to a 12:12-h daylight/darkness and allowed unlimited access to chow and water. They were divided into 6 groups as follow:

Group 1 (Sham group), where 10 rats were subjected to right nephrectomy without exposure to left renal pedicle ischemia.

(Group 2) Positive control group, where 10 rats were subjected to right nephrectomy and left renal ischemia where renal pedicles were clamped with atraumatic vascular clamps for 60 minutes. Then the vascular clamps were released to allow the reperfusion of the ischemic kidney.

(Group 3) Treated 1 group, where 10 rats were subjected to right nephrectomy and left renal ischemia where renal pedicles were clamped with atraumatic vascular clamps for 60 minutes. Then the vascular clamp were released to allow the reperfusion of the ischemic kidney. This group was received DPPD (0.5 g / kg, i.p.) 24 hours before the induction of ARF.

(Group 4) Treated 2 group, where 10 rats were subjected to right nephrectomy and left renal ischemia where renal pedicles were clamped with atraumatic vascular clamps for 60 minutes. Then the vascular clamps were released to allow the reperfusion of the ischemic kidney. This group was received DPPD (0.5 g / kg, i.p.) 24 hours after the induction of ARF.

(Group 5) Treated 3 group, where 10 rats were subjected to right nephrectomy and left renal ischemia where renal pedicles were clamped with atraumatic vascular clamps for 60 minutes. Then the vascular clamps were released to allow the reperfusion of the ischemic kidney. This group was received DPPD (0.5 g / kg, i.p.) 48 hours after the induction of ARF.

(Group 6) Treated 4 group, where 10 rats were subjected to right nephrectomy and left renal ischemia where renal pedicles were clamped with atraumatic vascular clamps for 60 minutes. Then the vascular clamps were released to allow the reperfusion of the ischemic kidney. This group was received MSCs, which were processed and cultured for 14 days, in a dose of (106) by IV infusion at the rat tail vein 24 hours after the induction of ARF.

Blood samples were collected from the retro-orbital vein 4 days after operation. Sera were separated. The rats of all groups were sacrificed (by co2 narcosis) after 4 days of induction of

the acute renal failure to obtain renal tissue specimens (Kawai *et al*, 2009).

Protocol of MSCs isolation: Isolation of MSCs from rat bone marrow was performed as described previously by Mcfarlin *et al*., (McFarlin *et al*, 2006) and maintained in CO_2 incubator at 37ºC. Tissue culturing was maintained till passage 3. Mesenchymal stem cells were injected in rats through caudal veins 1×10^6 for each rat.

Investigations provided to measure renal injury: Rats were sacrificed to evaluate the severity of injury in each kidney. At the end point all rats were sacrificed under anesthesia induced with phenobarbital sodium injection (50 mg/kg body weight, intraperitoneal). Kidneys were removed, cut transversely into 3 slices. One part was embed in Tissue Tek (OCT compound) for immunofluorescent study, the second part was snap-frozen in liquid nitrogen for future genetic study and the last slice was fixed in 10% buffered formalin, and embedded in paraffin for morphological studies (Yamagishi H *et al*, 2001; Zahran F *et al*, 2014).

Biochemical examination of blood and tissue

- Blood samples were used for determination of serum creatinine, BUN levels and NAG.
- Tissue samples were used for determination of malondialdehyde (MDA), superoxide dismutase, glutathione peroxidase, reduced glutathione and hydroxyproline. These parameters were measured using an automated spectrophotometer (Slim Plus, Italy) and microplate Reader (Stat Fax 3200, Awareness).

Analysis of kidney histopathology

Serial 4-µm sections of the cortex and the medulla of the kidney was stained with hematoxylin and eosin (H&E) and Masson trichrome for pathological examination.

Quantitative determination of superoxide dismutase in tissue extract. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye (55Nishikimi M *et al*, 1972).

Quantitative determination of reduced glutathione (GSH) in tissue extract. This method based on the reduction of 5 5'-dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm (Beultler E *et al*, 1963).

Quantitative determination of MDA in tissue extract. MDA is the end product of fatty acid peroxidation reacts with thiobarbaturic acid in acidic medium at temperature of 95 Celsius and form a colored complex (Ohkawa H *et al*, 1979).

Histopathological examination: Kidneys were perfused in a retrograde fashion through the abdominal aorta using saline 0.9% till complete clearance of the perfusion fluid, and then 10% neutral buffered formalin for in situ fixation. Both kidneys in all groups were harvested, cut longitudinally, and send for pathological evaluation in 10% neutral buffered formalin. Samples were processed and embedded in paraffin wax and sections (4 µm thick) were evaluated for the following:

Tubular injury score. Sections were stained with H & E for light microscopic examination. In the cisplatin-induced ARF model, tubular injury is most evident in the outer stripe of the outer medulla (OSOM). Thus, the degree of proximal tubular injury in this area was assessed and quantified in accordance with the scoring system modified by (Kinomura *et al*., 2008). Twenty randomly selected fields were observed at a magnification of x400. The degree of tubular injury was quantified as a score between 0 and 5 as the following: 0: normal; 1: tubular cells exhibiting desquamation from the tubular basement membrane (TBM), swelling, vacuolar degeneration and necrosis involving <20% of the tubules; 2: 20-40% tubules are involved; 3: 40-60%; 4: 60-80%; and 5: 80-100%.

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 17.0 to obtain descriptive data. Descriptive statistics were calculated in the form of mean ± standard deviation (SD) and median, minimum and maximum. In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests: ANOVA (analysis of variance): Used to compare between more than two groups of numerical (parametric) data followed by post-hoc tukey, Kruskal Wallis test: Used to compare between more than two groups of numerical (non-parametric) data followed by mann-whitney for pairwise comparisons or Repeated measures ANOVA (analysis of variance): Used to compare between more than two related groups of numerical (parametric) data followed by post-hoc LSD. A P value <0.05 was considered statistically significant.

RESULTS

Laboratory biochemical parameters

The present study involved 6 groups and the results of studied parameters of these groups were analyzed and presented in tables and in figures

Serum creatinine in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum creatinine compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant decrease in serum creatinine compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum creatinine compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum creatinine compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant decrease in serum creatinine compered to groups that received antioxidant 24,48 hours after the induction of ARF ($p < 0.001$).

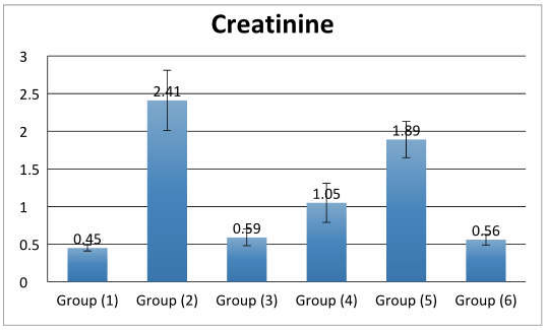

Fig 1 Effect of antioxidant and mesenchymal stem cell therapy on serum creatinine (mg/dl) in rat acute renal failure model induced by ischemia-reperfusion

Creatinine clearance in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in creatinine clearance compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant increase in creatinine clearance compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in creatinine clearance compared to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant decrease in creatinine clearance compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant increase in creatinine clearance compared to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

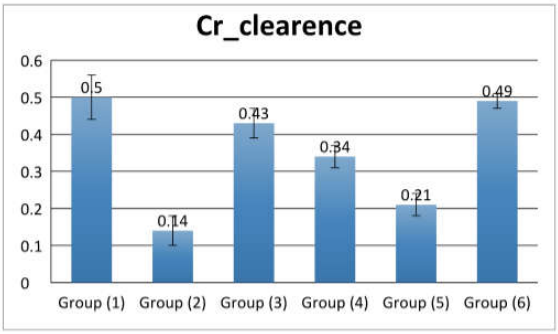

Fig 2 Effect of antioxidant and mesenchymal stem cell therapy on on creatinine clearance (ml/min./100g body weight) in rat acute renal failure model induced by ischemia-reperfusion

Serum BUN in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum BUN compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant decrease in serum BUN compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum BUN compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant increase in serum BUN compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant decrease in serum BUN compered to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

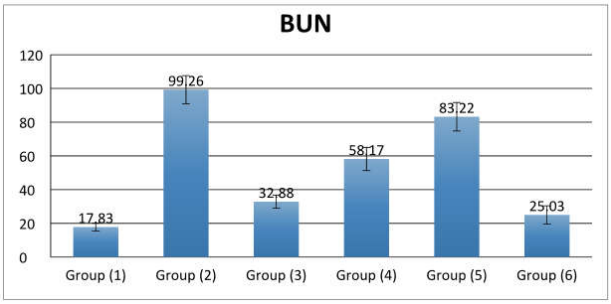

Fig 3 Effect of antioxidant and mesenchymal stem cell therapy on serum BUN (mg/dl) in rat acute renal failure model induced by ischemia-reperfusion

Urinary Total Protein in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in urinary total protein compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant decrease in urinary total protein compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in urinary total protein compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant increase in urinary total protein compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant decrease in urinary total protein compered to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

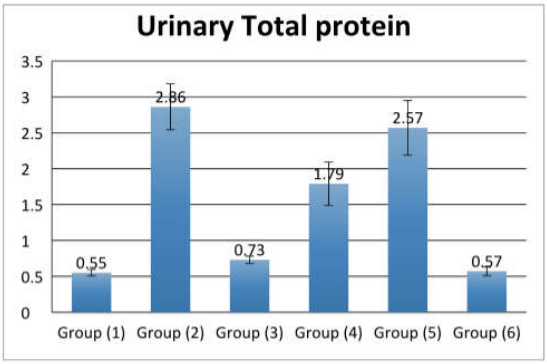

Fig 4 Effect of antioxidant and mesenchymal stem cell therapy on total protein in urine (mg/dl) in rat acute renal failure model induced by ischemia-reperfusion

N-Acetylglucosaminidase in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in NAG compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant decrease in NAG compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in NAG compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant increase in NAG compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant decrease in NAG compered to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

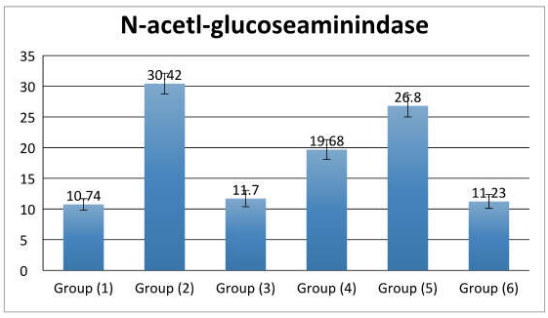

Fig 5 Effect of antioxidant and mesenchymal stem cell therapy on N-acetyl- glucosaminidase (mU/ml) in rat acute renal failure model induced by ischemia-reperfusion

Superoxide dismutase in tissue extract in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in SOD compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant increase in SOD compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in SOD compared to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant decrease in SOD compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant increase in SOD compared to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

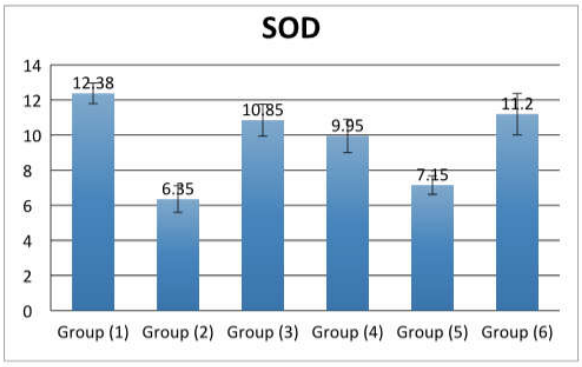

Fig 6 Effect of antioxidant and mesenchymal stem cell therapy on superoxide dismutase in tissue extract (U / mg protein) in rat acute renal failure model induced by ischemia-reperfusion

Glutathione reduced in tissue extract in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in glutathione reduced compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant increase in glutathione reduced compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant decrease in glutathione reduced compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant decrease in glutathione reduced compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant increase in glutathione reduced compared to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$)

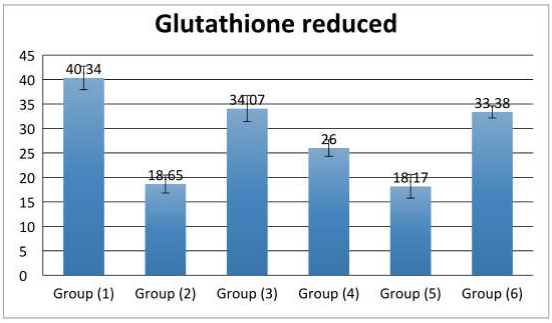

Fig 7 Effect of antioxidant and mesenchymal stem cell therapy on glutathione reduced in tissue extract (µmol/g protein) in rat fibrosis in rat acute renal failure model induced by ischemia-reperfusion.

Malondialdehyde in tissue extract in all studied groups

Positive control group, group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in MDA compered to sham group ($p < 0.001$) but both stem cells group and group that received antioxidant 24 hours before the induction of ARF showed no significant change compared to control group. While, all treated groups showed significant decrease in MDA compared to positive control group ($p < 0.001$).

Group that received antioxidant 24 hours after the induction of ARF and group that received antioxidant 48 hours after the induction of ARF showed significant increase in MDA compered to group that received antioxidant 24 hours before the induction of ARF ($p < 0.001$) but stem cells group showed no significant change compared to group that received antioxidant 24 hours before the induction of ARF.

Group that received antioxidant 48 hours after the induction of ARF showed significant increase in MDA compered to group that received antioxidant 24 hours after the induction of ARF ($p < 0.001$). While, stem cells group showed significant decrease in MDA compered to groups that received antioxidant 24, 48 hours after the induction of ARF ($p < 0.001$).

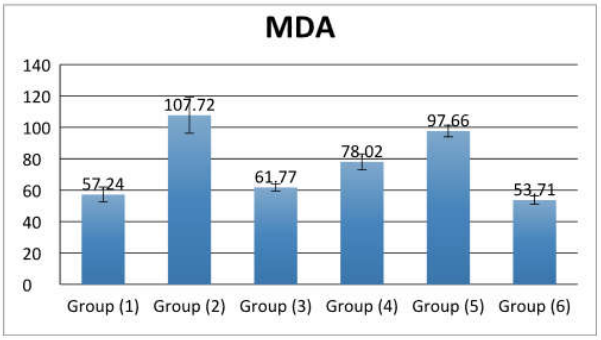

Fig 8 Effect of antioxidant and mesenchymal stem cell therapy on MDA in tissue extract (nmol /g tissue) in rat acute renal failure model induced by ischemia-reperfusion

Histopathological examination
Tubular injury score

The kidney specimens obtained from rats in sham (negative control) group showed normal kidney architecture without any abnormality in renal tubules and glomeruli (fig.9). Kidney specimens obtained from rats in positive control group showed marked disturbed kidney architecture in the form of shrinkage of vascular tuft, thrombosis of glomerular capillaries, disrupted glomerular basement membrane (glomerular necrosis), marked tubular atrophy with tubular necrosis, interstitial haemorhage, fibrosis and inflammation with normal blood vessels. (fig.10).

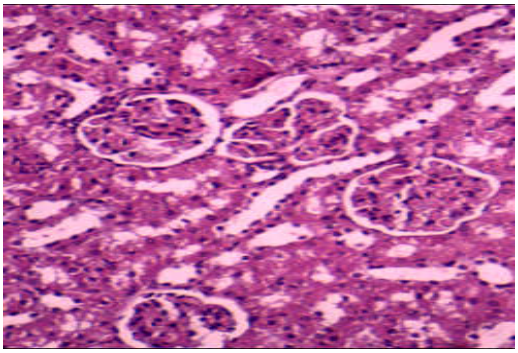

Fig 9 kidney specimens with normal architecture (normal glomeruli and renal tubules (sham group) (magnification 100x). (GRADE :0)

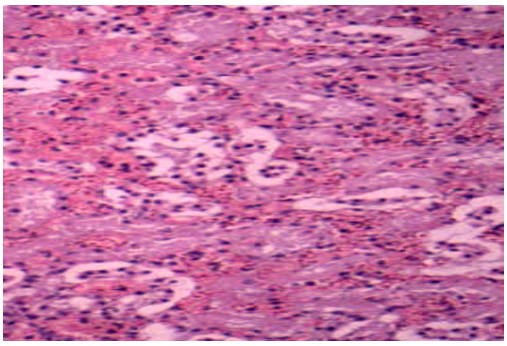

Fig 10 kidney specimens showing marked thrombosis in glomerular capillaries with disrupted glomerular basement membrane, Leukocytic infiltration (glomerular necrosis), marked tubular necrosis, interstitial haemorhage and inflammation and normal blood vessels. (Positive control) Magnification a= 100x. (GRADE :5)

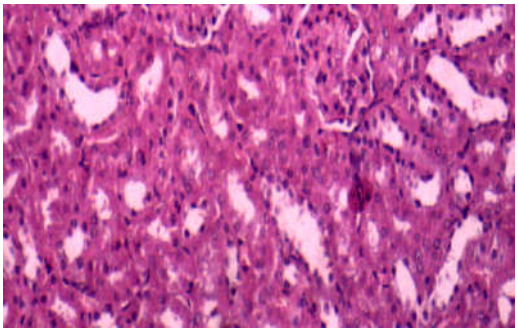

Fig 11 kidney specimens with normal architecture (normal glomeruli and renal tubules (stem cells group) (magnification 100x). (GRADE :0)

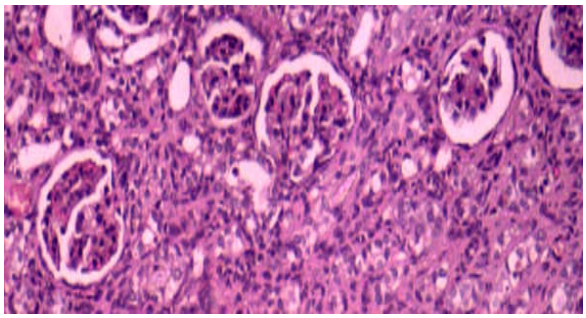

Fig 12 kidney specimens showing regeneration of the lumen, less tubular atrophy, very mild interstitial fibrosis and normal blood vessels. (Antioxidant 24 hr before operation) Magnification a= 100x. (GRADE:1)

Kidney specimens obtained from rats treated with MSC showed normal kidney architecture without any abnormality in renal tubules and glomeruli (fig. 11). While kidney specimens obtained from rats treated with DPPD 24 hr before operation showed regeneration of the lumen, less tubular atrophy, very mild interstitial fibrosis and normal blood vessels. (fig.12).

Kidney specimens obtained from rats treated with DPPD 24 hr after operation showed normal regarding messangium and vascular tuft, mild interstitial fibrosis and normal blood vessels. (fig. 13). While kidney specimens obtained from rats treated with DPPD 48 hr after operation showed mild shrinkage of vascular tuft with normal basement membrane and cellularity, marked tubular atrophy with cast formation, mild interstitial fibrosis and normal blood vessels. (fig.14).

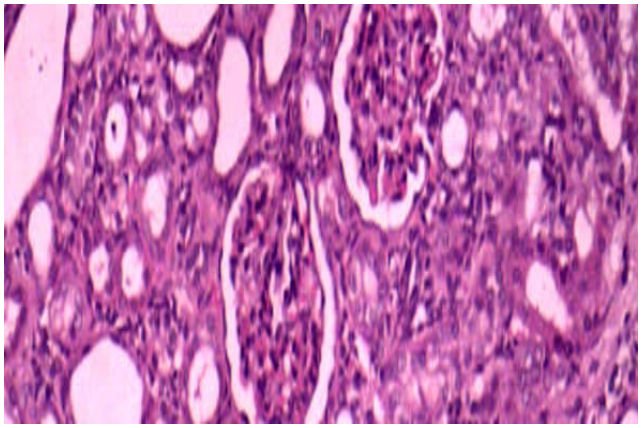

Fig 13 kidney specimens showing normal regarding messangium and vascular tuft, mild interstitial fibrosis and normal blood vessels. (Antioxidant 24 hr after operation) Magnification a= 100x. (GRADE :2)

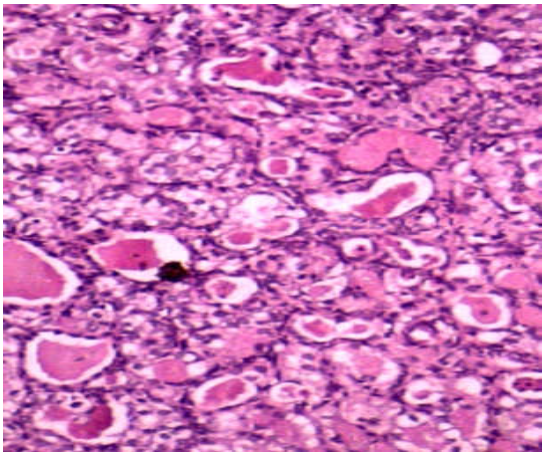

Fig 14 kidney specimens showing showed mild shrinkage of vascular tuft with normal basement membrane and cellularity, marked tubular atrophy with cast formation, mild interstitial fibrosis and normal blood vessels.

Discution

Although the clinical management of AKI patients has significantly improved in recent years, we still lack specific therapies to enhance kidney repair. Recovery after acute injury is critical for patient morbidity and mortality in the hospital setting (Silver *et al*, 2015).

The pathophysiological process of AKI following IRI leads to functional and structural changes that are centered around the proximal tubule cells and endothelium (Lameire *et al*, 2006).

Renal ischemia induces endothelial stress, which leads to swelling of the renal vasculature and consequent narrowing of the lumen with no-reflow phenomenon following the restoration of the renal blood flow (Lameire *et al*, 2006). Moreover, the constant communication between the tubular epithelium and the vascular endothelium via 'cellular cross-talk' plays a role in the pathogenesis of AKI associated with IRI (Lameire *et al*, 2006).

When exposed to IRI the renal tubular epithelium generates mediators such as tumor necrosis factor (TNF)-α, transforming growth factor-β and interleukin (IL)-6 that may affect the endothelium directly or by the potentiation of an inflammatory response on the other hand, IRI stimulates oxidative stress in renal slices results in liberation of destructive free radicals and hydroxyl anions leading to tubular atrophy (Lameire *et al*, 2006).

Although the clinical management of patients with AKI has significantly improved in recent years, specific therapies to enhance kidney repair are lacking. Recovery following acute injury is critical for minimizing patient morbidity and mortality in the hospital setting (Bonventre *et al*, 2011).

The emerging field of regenerative medicine is progressing rapidly and is supported by a large number of studies demonstrating that stem cells have the capacity to substitute for damaged or lost differentiated cells in various organs and tissues (Zhuo *et al*, 2011; Paglia *et al*, 1967).

(Chen Y *et al*., 2011) demonstrated an anti-inflammatory pattern in MSC-treated animals, indicating the potential of MSCs to modulate IRI, leading to the earlier regeneration of damaged renal tissue.

Recent studies showed the marvelous role of antioxidants as a prophylactic agent against acute renal injury by trapping of destructive free radicals and dampen inflammation (Liu *et al*, 2013).

The aim of the present study was to address the role of MSCs and antioxidant named DPPD in AKI secondary to renal IRI. In this study, one million MSCs were injected into the rats 24 hours after the induction of ARF modeling procedure, and other 3 groups of rats were received DPPD one day before, one day after and two days after AKI. The surgery entailed clamping of the renal pedicle for 60 min in anesthetized rats to cause severe IRI injury in the kidney.

The damaging effects of renal IRI comprise a complex interrelated sequence of events, eventually resulting in both apoptosis and necrosis of the renal cells (Sivarajah *et al*, 2003). Thus an ideal modality to manage IRI injury should work by multiple mechanisms.

In the present study, the use of MSCs and antioxidant was found to be capable of ameliorating renal dysfunction, as demonstrated by improvements of serum creatinine levels, BUN, creatinine clearance, total protein in urine, NAG and serum magnesium and improvement of the histological indices of injury in the renal cortex and outer medulla. Moreover, the use of MSCs and antioxidant partially ameliorated oxidative stress and lipid peroxidation as reflected by changes in the levels of MDA, SOD and glutathione reduced in renal tissue. But stem cells showed the more effective role in renal

protection, also using antioxidant in early stages showed better effect. The results are concordant with the results of previous studies.

(Chen Y *et al*., 2011) demonstrated that MSCs therapy minimized kidney injury following IRI by suppressing oxidative stress and the inflammatory response. (Zhuo *et al*, 2011) also showed that MSCs were able to ameliorate AKI induced secondary to IRI in a mouse model via the suppression of cytokines (IL-1β and TNF-α) and chemokines (microphage inflammatory protein-1α), which led to an anti-inflammatory activity and alleviation of tubular necrosis. Based on the ability of cyclosporine to reduce the generation of reactive oxygen species (Zhuo *et al*, 2011) administered cyclosporine in addition to MSCs to mitigate AKI in the setting of IRI in rats, and this combined treatment showed an improved protective effect against acute IRI compared with either treatment alone.
By contrast, in a study conducted in a mouse model by (Jiang *et al*, 2007) it was concluded that transplantation using hematopoietic stem cells did not improve the renal repair process. In addition, the use of stem cells is currently limited by the possible risks associated with the use of stem cell therapy, and controversy exists regarding the exact mechanism underlying the effects of the therapy; thorough scientific exploration is required to assess mechanism, safety profile, reproducibility and methods for monitoring the administered stem cells (Semedo *et al*, 2007).

Notably, MSCs and (DPPD before operation) exhibit both an early anti-acute insult effect and late regenerative activities, as demonstrated by the results of the present study. Indication of a significantly intact renal clearance, creatinine, BUN, NAG and serum magnesium in the MSCs and (DPPD before operation) groups following renal ischemia-reperfusion, as compared with that of the untreated positive control and other antioxidant groups. This observation, combined with a notably lower level of MDA and increase levels of SOD and glutathione reduced in the renal tissue is indicative of an early protective mechanism of MSCs and DPPD, which may act through antioxidative activity with a consequent anti-inflammatory effect. The histopathological evaluations confirm this finding, as indicated by the significantly lower injury score for the outer stripe of the outer medulla scoring.

The late regenerative stimulant activity of DPPD (24, 48 hr after AKI) is indicated by the markedly small significant improvement in NAG and kidney functions measurement with small proportional improvement of the tissue MDA, SOD and gluatathione levels, together with a significant improvement in the regenerative score of the OSOM in comparison with the score of positive group.

In conclusion, the results of the present study demonstrate the ability of MSCs and early administration of antioxidant to ameliorate the renal injury and dysfunction associated with IRI in rats by early protective antioxidative activity and later regenerative stimulant activity. However, the major limitations of this study are that molecular studies of the renal tissues were not conducted, and the underlying mechanisms involved in the therapeutic effect of MSCs and DPPD against renal IRI remain descriptive. Further investigations, therefore, are warranted to clarify the exact mechanisms underlying the effects of the stem cells and antioxidants. Since satisfactory treatment modalities for renal IRI are currently lacking, stem cell therapy may be promising provided that gaps in knowledge are filled to assure a safe transition to use in humans.

CONCLUSIONS

We concluded that either mesenchymal stem cells or antioxidant had significantly ameliorated the cisplatin induced nephrotoxicity via their anti-inflammatory, anti-oxidative stress and anti-fibrotic potentials. However more studies are still needed to solidify our findings and to investigate other possible underlying nephroprotective mechanisms of MSCs and antioxidants.

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