

The biochemical effect of probiotic and/or mesenchymal stem cells on LPS-induced kidney disorder

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Abstract

The current study aimed to evaluate the beneficial effect of kefir probiotic and/or Mesenchymal stem cells (MSCs) on acute kidney injury (AKI) induced by lipopolysaccharide (LPS) challenge, and to investigate could kefir potentiate the therapeutic action of MSCs. Sixty female albino rats were used in this study and divided into 6 groups (10 rats each): control group; LPS-challenged group; LPS+ MSCs group; LPS + kefir group; kefir +LPS +kefir (prophylactic) group and kefir+ LPS + MSCs + kefir (prophylactic-MSCs) group. Samples were collected at two point's time. Renal function, serum IL-10, TNF- α , renal MDA, GSH contents, SOD and PON-I were assayed. Their RNA expression of NF- κ B, iNOS and caspase-3 were monitored in kidney tissue. Our results revealed that LPS significantly increased renal function tests, TNF- α in association with dramatic decrease of creatinine clearance and serum IL-10 levels. Oxidative stress was proved in LPS group by increasing MDA level, reduction in GSH content, SOD and PON-1 activities in kidney. The mRNA expression of NF- κ B, iNOS and caspase-3 were significantly up-regulated in AKI. Administration of kefir or MSCs alone significantly attenuated LPS-induced AKI. The pre and co-treatment of kefir with MSCs potentiate their therapeutic action. Conclusion: A combination of kefir probiotic and MSCs may be of interest for clinical use.

Keywords: kidney; lipopolysaccharide; kefir; MSCs; oxidative stress

1. Introduction

Sepsis is characterized by the systemic inflammatory response to infection and is associated with multiply organ failure [1]. Sepsis – induced acute kidney injury (AKI) is associated with 70% of mortality, and is a serious medical problem in the intensive care Unit [2]. Acute renal injury (AKI) is characterized by a rapid, potential reversible decline in renal function and retention of nitrogenous waste products over a period of hours or days [3].

The exposure of renal cells to lipopolysaccharides (LPS) induced inflammatory response and free radicals including reactive oxygen species (ROS) and nitrogen oxide generation[2]. LPS activates the nuclear factor kappa B (NF- κ B) pathway in the kidney leading to inflammatory cytokine synthesis, free radical production and cell death [4]. In sepsis-induced AKI, tumor necrosis factor (TNF- α) magnifies the responses mediated by other cytokines and free radical generation through the activation of nuclear factor-kappa B signaling pathway[5, 6].

Stem cell therapy holds a great promise for the repair of injured tissues including the kidney. Stem cells are undifferentiated cell that undergo both self-renewal and differentiation into one or more cell types [7]. There has been considerable focus on the ability of stem cells to differentiate into non-hematopoietic cells of various tissues Lineages, including the kidney [8]. This growing evidence has led to a reconsideration of the source of cells contributing to renal repair following injury [9].

The mechanism of action of stem cell therapy is investigation in most disease conditions; very low level engraftment of circulating bone marrow derived stem cells has been shown [10]. The percentage of incorporated stem cells varies widely, but it is usually below 1% in a given organ, and in addition, its magnitude depends on the studied disease model [9]. Other mechanistic possibilities for the therapeutic effects of stem cells include fusion with resident organ cells [11], immune modulation [12] and paracrine mechanisms elicited through trophic mediators [13] that result in the inhibition of fibrosis and apoptosis.

Kefir is a nature probiotic that contains live bacteria, which are beneficial to health [14]. Kefir is an acidic and mildly alcoholic fermented milk product that is believed to contain many functional substances [15] and different from other milk products in that it is not a result of the metabolic activity of a single species of microflora, but it is the product of fermentation with a mixed group of microflora confined to a matrix of discrete "Kefir grains" which recovered after fermentation [16]. Kefir has been considered a probiotic due to its antioxidant and anti-inflammatory properties [17,18].

This probiotic has been proposed to contribute to reduce the progressing of renal injury in diabetes [19,20].

This study was conducted to study the effect of MSCs as a line of treatment for AKI induced by LPS. Also to investigate the effect of kefir on the production of oxidative stress, inflammation in AKI and finally to study the hypothesis that could kefir probiotic potentiate the therapeutic action of MSCs.

2. Materials and Methods

2.1. Preparation of bone marrow (BM)-derived mesenchymal stem cells from rats

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old female white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and re-suspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO₂ for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were re-suspended in serum supplemented medium and incubated in 50 cm² culture flasks (Falcon). The resulting cultures were referred to as first-passage cultures[21]. Cells were identified as being MSCs by their morphology, adherence and their power to differentiate into osteocytes[22] and chondrocytes [23].

2.2. Preparation of milk kefir

Kefir was purchased from organic culture (Buckly'S USA). Freeze-dried kefir grains was added to pasteurized milk (20 mg/dl) and inoculation at 23°C for 16 h, when the desired pH was reached the fermentation was stopped by cooling the flask in ice bath and storing at 4°C until utilization[24].

2.3. Animals and Experimental design

Sixty female white Albino rats of an average weight of 150-170 g 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. This study was approved by the local ethics committee of NODCAR. The rats were divided into 6 groups (10 rats each) as follows:

Group1 (control): Animals of this group received a single intraperitoneal injection of sterile saline-PBS (pH 7.4) as a vehicle.

Group 2 (LPS-induced AKI): Rats of this group were received a single intraperitoneal injection of lipopolysaccharide (LPS, 10 mg / kg b. wt. in 1 ml of sterile PBS (pH 7.4) to induce AKI [25]. LPS (extracted from *Escherichia Coli* 0111:B4) was purchased from Sigma-Aldrich (St Louis, MO).

Group 3 (LPS + MSCs): Rats of this group were intraperitoneally injected with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, then rats were received a single intravenous injection of MSCs in the tail vein 30 minutes after LPS injection (MSCs were processed and cultured for 14 days, in a dose 3×10⁶ cells / rat)[21].

Group 4 (LPS + kefir): Animals of this group intraperitoneally were injected with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, then 30 minutes after kefir was administered for 7 days by gavage, at a dose of 1.8 ml / rat/day[19].

Group 5 (kefir +LPS +kefir, prophylactic): Rats of this group were intraperitoneally injected with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, rats of this group pretreated with kefir for 7 days before LPS injection and continue for 7 days later.

Group 6 (kefir+ LPS + MSCs + kefir, prophylactic-MSCs): The rats of this group were intraperitoneally injected with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, and treated with MSCs (3×10⁶cells/ rat as a single dose)[21]by IV infusion at the rat tail vein 30 minutes after LPS injection, rats of this group pretreated with kefir for 7 days prior and post LPS injection.

Urine was gathered via metabolic cages and the supernatant was obtained and collected. Blood samples were collected from the retro orbital vein. Sera and urine were separated and used for biochemical analysis. At the end of experiment, kidneys of decapitated rats were removed quickly, washed with cold saline and used for biochemical estimation.

2.4. Biochemical Analysis

2.4.1. Assessment of renal function:

Renal function, in the form of serum urea, creatinine concentration (in serum and urine) and urinary albumin were assayed by using a commercially available kits supplied by BioSystems S.A., Spain [26,27]. Creatinine clearance (Cr_{Cl}) was calculated by determining creatinine concentration in timed urine collections and simultaneously in blood using the following equation: Cr_{Cl}(ml/min) = [urinary creatinine (mg/dl) x urine volume (ml/min)] / [serum creatinine (mg/dl)][28].

2.4.2. Estimation of serum levels of interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α):

Serum levels of rat IL-10 and TNF- α were assayed by enzyme-linked immunosorbent assay kits supplied by Quantikine, R&D Systems Inc., Minneapolis, MN, USA according to the instructions of manufacturers.

2.4.3. Measurement of renal malondialdehyde (MDA) level:

The method involved heating of homogenized kidney samples with thiobarbituric acid substance (TBA) reagent for 20 min in a boiling water bath. After cooling, the solution was centrifuged and the absorbance of the supernatant was determined at 532 nm according to Wills [29] to measure the MDA concentration compared to 1, 1, 3, 3, tetraethoxypropane as standard.

2.4.4. Measurement of kidney reduced glutathione (GSH) content:

The method involved protein precipitation from homogenized kidney samples after centrifugation, 5, 5-dithiobisnitrobenzic acid (DTNB) solution was added to the supernatants, the colored was rapidly developed and the absorbance was measured at 412 nm [30] compared to GSH standard curve.

2.4.5. Measurement of kidney superoxide dismutase (SOD) activity:

SOD activity in kidneys homogenate was measured through the inhibition of nitrobluetetrazolium (NBT) reduction by O²-generated by the xanthine/xanthine oxidase system. One SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1 ml reaction solution per milligram tissue protein and the result was expressed as U/mg protein [31].

2.4.6. Measurement of kidney paraoxonase-1(PON-1) activity:

PON-1 enzyme activity of kidney tissues was measured by recording the initial rate of paraxon hydrolysis as a substrate to p-nitrophenol at 405 nm, A PON-1 activity of one U/mg protein was defined as 1 μ mol p-nitrophenol formed per minute per mg protein[32].

2.5. Quantitative real time PCR for NF- κ B, iNOS and caspase-3 gene expression in liver tissue:

Frozen kidney tissue was homogenized in trizol reagent. Total RNA was extracted and purified using RNeasy purification reagent (Qiagen, Valencia, CA). cDNA was generated from 5 μ g of total RNA extracted with 1 μ l (20 pmol) antisense primer and 0.8 μ l superscript AMV reverse transcriptase for 60 min at 37 °C. The relative abundance of mRNA species was assessed on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from Gene Bank (Table 1). All primer sets had a calculated annealing temperature of 60°C. Quantitative real time PCR was performed in duplicate in a 25- μ l reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2–3 μ l of cDNA. Amplification conditions were 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 sec and annealing/extension at 60°C for 10 min. Data from real-time assays were calculated using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of iNOS, caspase-3 and NF- κ B mRNA was calculated using the comparative threshold cycle method (Ct). All values were normalized to the β -actin gene [33].

Table 1: Sequence of the primers used for real-time PCR

Gene	Primer sequences
NF- κ B	Forward primer: 5'-GCTTACGGTGGGATTGCATT-3' Reverse primer: 5'-TTATGGTGCCATGGGTGATG-3'
iNOS	Forward primer : 5'-GCAGGATCAGAACACAGCAA3' Reverse primer : 5'-ATGGTTACGGGAGGGGTAAG 3'
Caspase-3	Forward primer: 5'- ATGGACAACAACGAAACCTC-3' Reverse primer: 5'- TTAGTGATAAAAGTACAGTTCCT-3'
β -actin	Forward primer :5'-CCAGGCTGGATTGCAGTT'-3 Reverse primer: '5-GATCACGAGGTCAGGAGATG'-3

2.6. Statistical Analysis

The results were expressed as the mean \pm standard error (SE) for five animals in each group. One-way analysis of variance (ANOVA) was used to compare group variables followed by a post hoc Duncan's test; P< 0.05 was considered significant. Data were statistically analyzed using the statistical package for social science 21 software (SPSS, Chicago, IL, USA).

3. Results

3.1 Effect of kefir and/ or MSCs on kidney disorder marker:

LPS induced significant elevations in serum levels of Creatinine, urea and urine albumin. These elevations were significantly maintained with time extension (Fig. 1). This rise in the examined renal marker was associated with significant reduction in Creatinine clearance when compared with the control non-treated group at the two examined time

points (Fig.1). On the other hand it was found that, treatment of intoxicated rats with kefir or MSCs alone significantly alleviate the toxic effect of LPS. The obtained results also revealed that, the pre and post administration of kefir to LPS challenged rats displayed a significant improvement in the examined parameter (Fig.1). A more pronounced improvement in the examined nephrotoxicity marker was obtained in the group of rats administered kefir for 7 days pre LPS injection and treated with MSCs in concomitant administration of kefir for other 7 days.

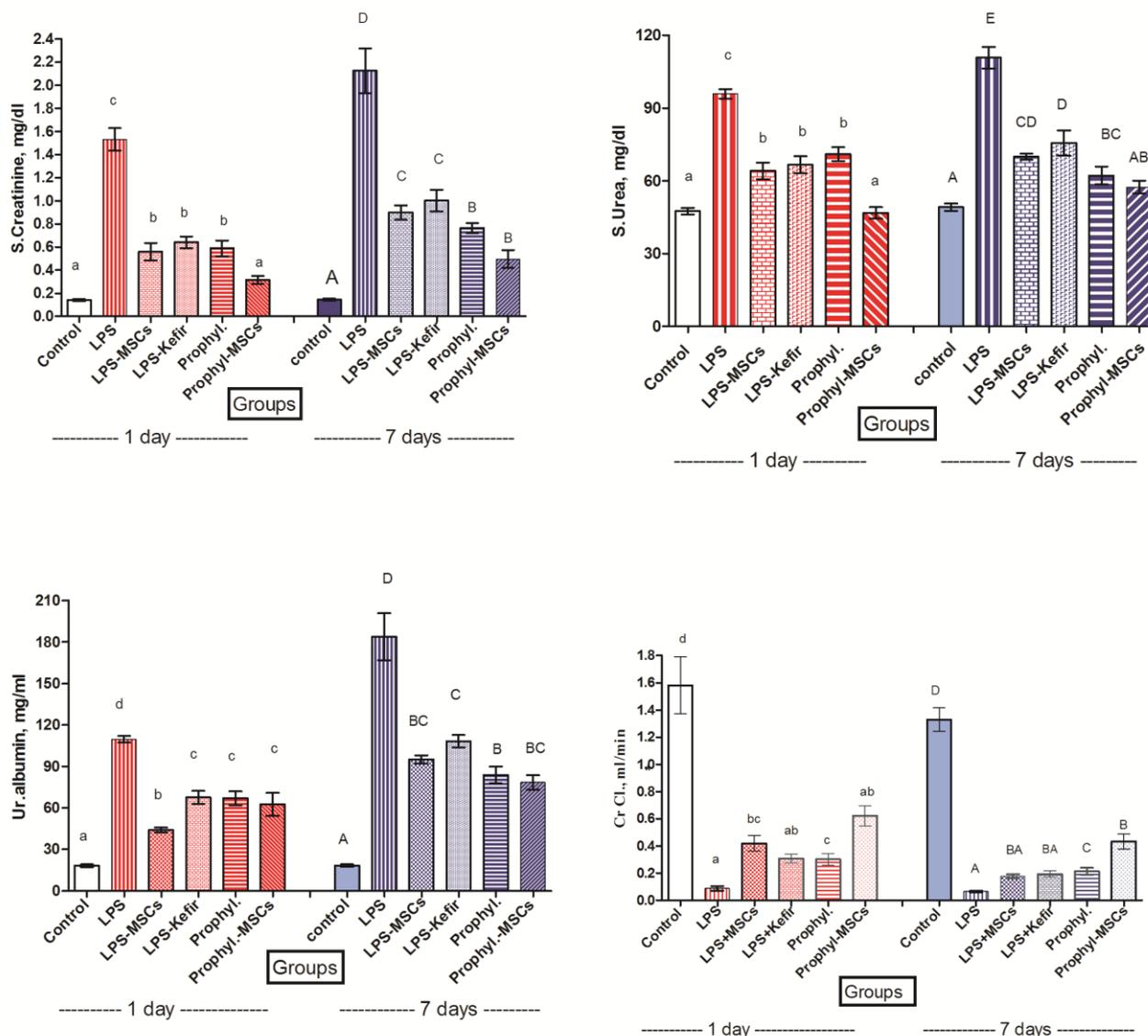


Fig. 1: Effect of probiotic and / or MSCs on kidney function of LPS-induced renal disorder at different time's point. Each value represents the mean of 5 rats ± SE. The different superscripts latter at the same time's point are significant at p<0.05.

3.2 Effect of Kefir and / or MSCs on circulating TNF-α and IL-10 in LPS challenged rats:

Fig. 2 represents serum TNF- α of all groups at the two examined time points. The obtained results revealed that, the serum levels of TNF- α were significantly elevated in all groups over the control non-treated group. Kefir and / or MSCs treated groups elicited a significant reduction in serum TNF-α compared to LPS injected rats. The obtained results also revealed that MSCs and kefir significantly attenuated the progressed increase in serum TNF-α level. LPS challenged rats pre and post treated with kefir and MSCs or rats treated either MSCs alone showed a more significant reductions in TNF-α levels compared to those treated with kefir alone (Fig. 2). On contrast serum levels of the anti – inflammatory cytokine IL-10 were significantly decreased in all examined group when compared with the control non- treated group (Fig. 2). All treatment regimens caused significant elevation in serum IL-10 levels. The obtained results also revealed that, the treatment of LPS challenged rats with kefir and MSCs enhanced the therapeutic capability of MSCs (Fig. 2).

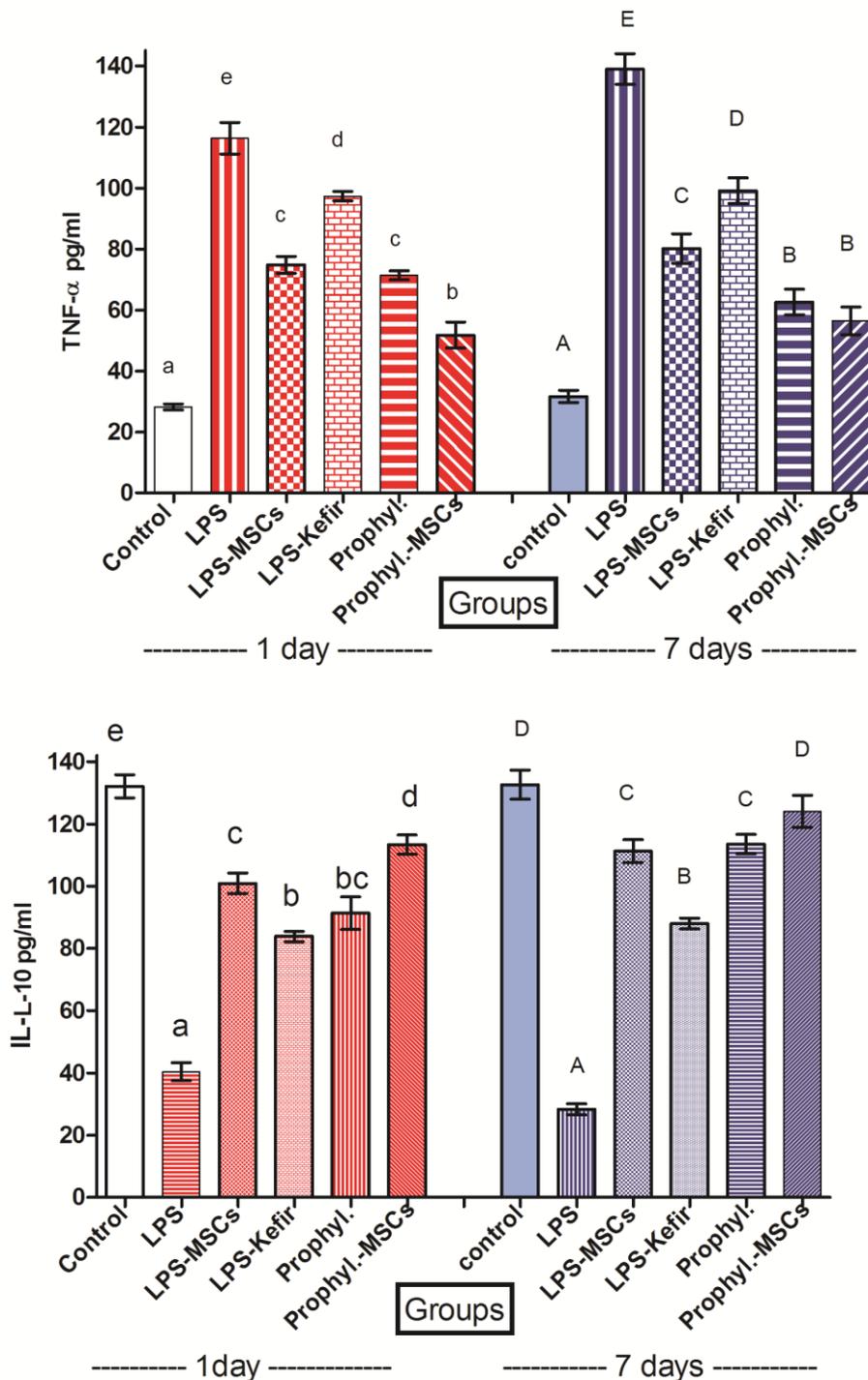


Fig. 2: Effect of probiotic and /or mesenchymal stem cells on TNF- α and IL-10 of LPS-induced renal disorder at different time's point. Each value represents the mean of 5 rats \pm SE. The different superscripts latter at the same time's point are significant at $p < 0.05$.

3.3 Effect of kefir and / or MSCs on renal GSH and MDA

Intoxication with LPS induced a significant decrease in renal tissue GSH content when measured at one and seven days after LPS-challenge (Fig.3). This reduction in renal GSH was associated with a marked elevation in lipid peroxidation index MDA concentration (Fig. 3). Administration of LPS challenged rats with kefir or MSCs alone caused a significant attenuation in LPS induced oxidative stress in kidney tissue. The obtained results clearly proved the ability of kefir probiotic to act as free radical scavenging agents.

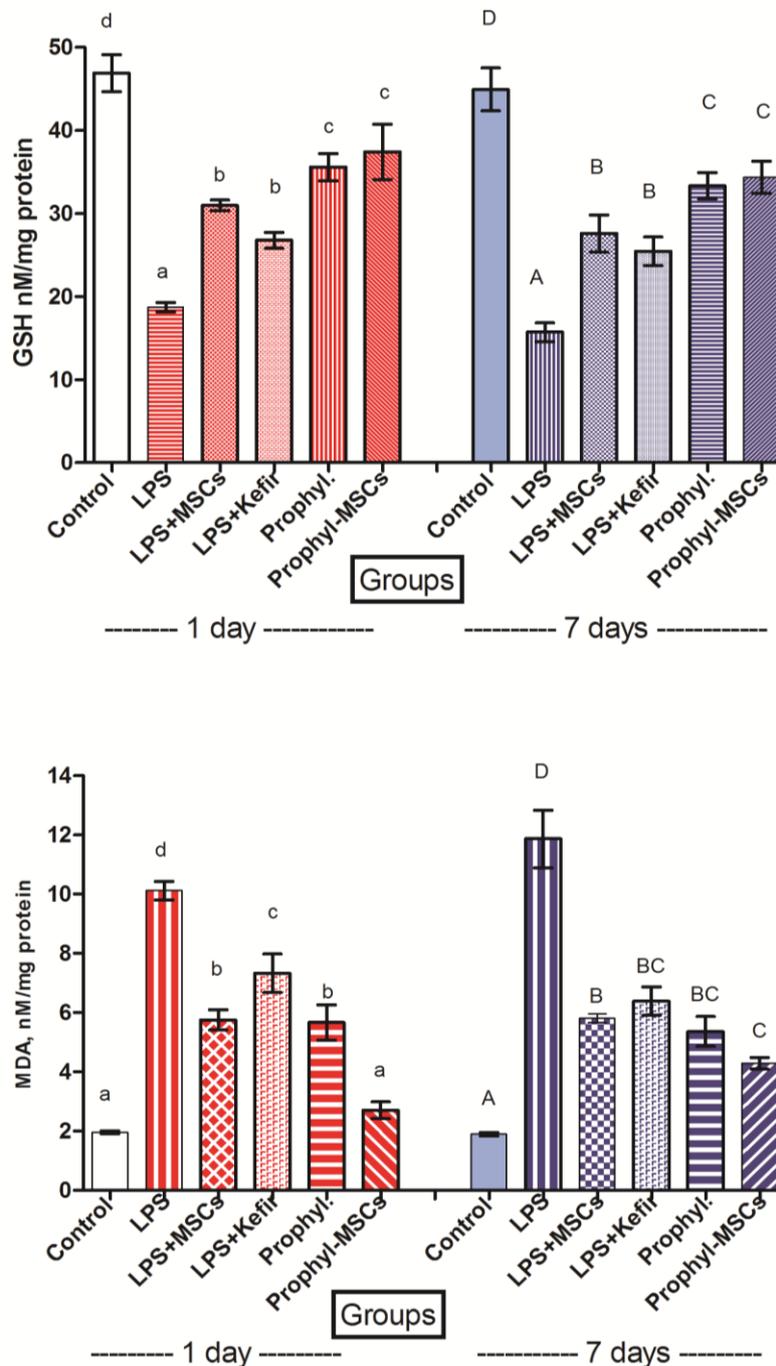


Fig. 3: Effect of probiotic and / or MSCs on kidneys GSH and MDA levels of LPS-induced renal disorder at different time's point. Each value represents the mean of 5 rats ± SE. The different superscript letter at the same time are significant at p<0.05.

The obtained results elicited the capability of MSCs restoring GSH kidney with combating the free radical production.

3.4 Effect of kefir and/or MSCs on renal antioxidant enzymes activity (SOD and PON-I):

Renal tissue SOD and PON-I were significantly reduced in LPS challenged rats when compared with control non treated group at one of the two examined time point (Fig 4).

An equivalent significant increase in SOD and PON-I activities was obtained when LPS-injected rats treated with kefir or MSCs alone (Fig. 4). The obtained results revealed that, treatment of LPS-challenged rats with kefir significantly potentiate the recovery of SOD and PON-I activities. The obtained results also revealed that, the administration of kefir before LPS challenge and its continuation with MSCs treatment elicited a more pronounced and protective effect against the progressed oxidation damage of LPS on renal tissue.

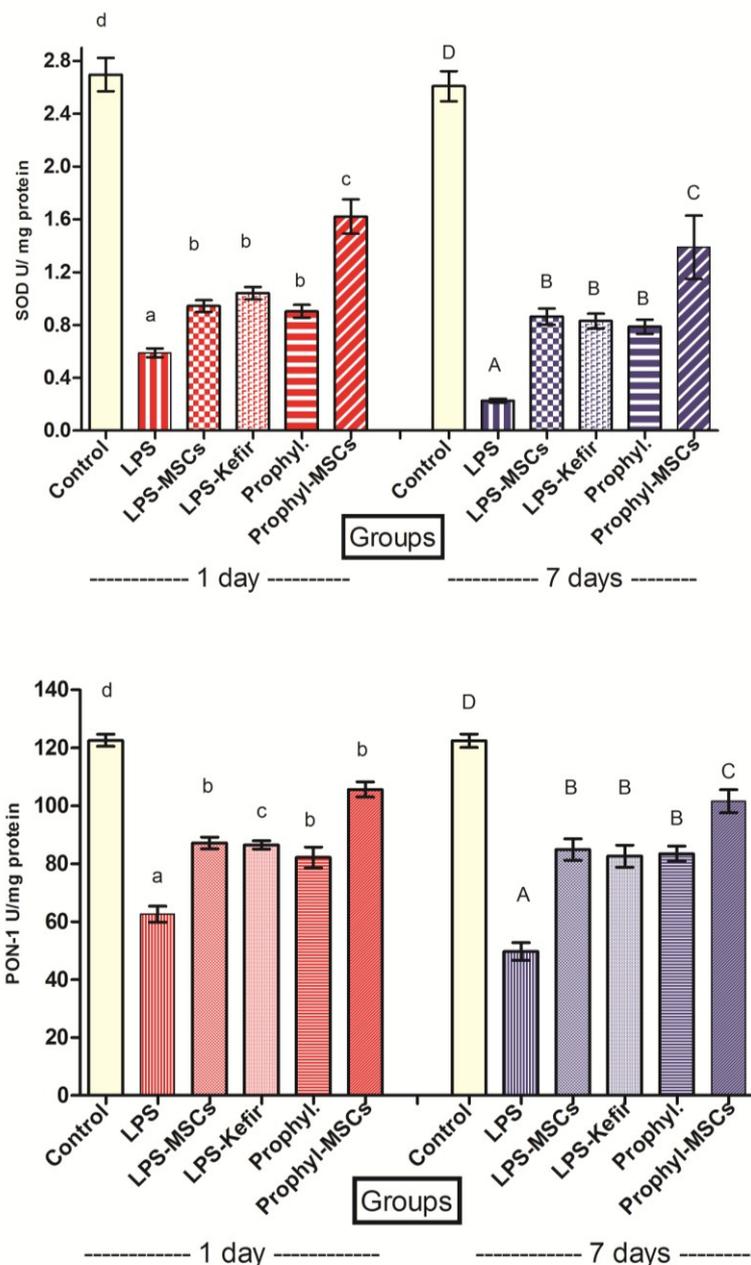


Fig. 4: Effect of probiotic and / or MSCs on kidneys SOD and PON-1 activities of LPS-induced renal disorder at different time's point. Each value represents the mean of 5 rats ± SE. The different superscripts latter at the same time's point are significant at p<0.05.

3.5. Effect of kefir and/or MSCs on renal tissue caspase-3, iNOS and NF-κB messenger RNA expression:

The obtained results of the quantitative real time PCR revealed that, kefir and/or MSCs significantly reduced LPS-induced apoptosis in renal tissue as indicated by the significant decrease in mRNA expression of caspase-3. The obtained results also revealed that, mRNA expression of caspase -3 decreased as kidney function was improved (Fig. 5).

There were significant differences between kefir and/or MSCs treated groups and LPS challenged rats at one day and 7 days of MSCs injection and kefir administration (Fig. 5).

The mRNA expression of iNOS was significantly reduced in kefir and/or MSCs treated group compared to LPS challenged rats (Fig. 5). These results indicated that Kefir – MSCs protection against renal apoptosis could be mediated by suppression of iNOS gene expression.

Fig. 5 showed that NF-κB mRNA expression gradually increased as time extended in LPS challenged rats. Administration of kefir and MSCs alone or in combination was significantly lower NF-κB mRNA expression. A more pronounced effect was obtained in the group of rats pre and post treated with kefir and MSCs (Fig. 5).

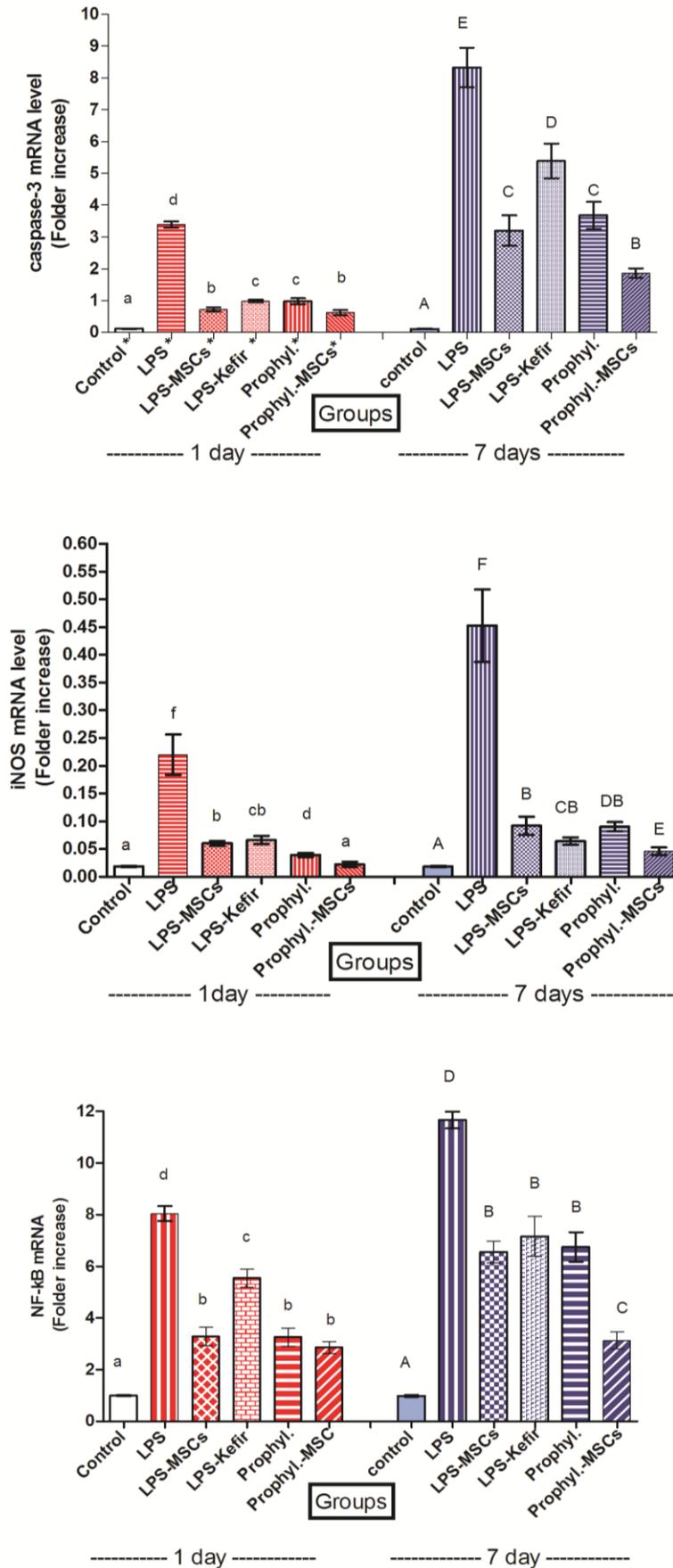


Fig. 5: Effect of probiotic and / or MSCs on kidneys Caspase-3, iNOS and NF-kB mRNA gene expression levels of LPS-induced renal disorder compared to Beta actin as an invariant internal control in real-time PCR assay. Each value represents the mean of 5 rats \pm SE. The different superscripts letter at the same time's point are significant at $p < 0.05$.

4. Discussion

LPS-induced renal failure in rats is a recognized experimental model that mimics the infection induced renal failure in humans [34-37]. In the present study, LPS challenged rats elicited a significant increase in serum creatinine and urea levels that associated with increased urine albumin and decreased creatinine clearance. The obtained increase in urinary protein in the current study is linked to the elevated blood urea which is known to be a function of or related to increased protein catabolism in mammals and / or the conversion of ammonia to urea as a result of increased arginase synthesis that involved in urea production [38]. In this study LPS challenge evoked the production of free radicals and generation of oxidative stress that evidenced through the increased concentration of renal MDA and decreased GSH with substantial decrease in the antioxidant enzymatic activity of renal SOD and PON-1. This decline in the SOD activity could be attributed to the reduction of renal tissue to counteract the consequence of the overproduction of reactive oxygen species in LPS challenged rats. PON-1 is an antioxidant that protects HDL and LDL from oxidation [39].

Kefir administration for 7 days both prior or post to LPS-challenge attenuated the increased serum creatinine, urea and urine albumin levels in association with improved creatinine clearance. Administration of kefir also restored of the SOD and PON-1 activities. The decreased content of ROS scavenger like GSH was also enhanced by kefir treatment both prior and post LPS-challenge. Kefir has also attenuates MDA production. These findings reflect the benefit role of kefir probiotic in protection of kidney tissue and proved the efficiency of kefir probiotic in preserving the cell membrane integrity of renal cells and support the results of Punaro *et al* [19] who attributed the efficiency of kefir in reducing progression of renal injury to its antioxidant properties. Liu *et al* [24] referred the antioxidant activity of kefir to its protein-donating power and SOD like activity. Moreover Guven *et al* [17] compared the anti-oxidative consequences of kefir and vitamin E against animal model of oxidative stress and reported that Kefir offers a more protection against oxidative change as compared to vitamin E.

Data of the current study confirmed that, MSCs transplantation in the animal in the MSCs treated groups provides the advanced in healing injury that associated with marked anti-per oxidative capability. MSCs transplantation significantly decreased LPS-induced raised of renal MDA with the elevation on GSH renal content. These findings proved the fact that MSCs are attractive candidates for renal repair [40]. The obtained increase in GSH content of renal tissue in MSCs treated groups is in the harmony with the work of Iyer *et al* [41] who reported that, the potential mechanism by which MSCs improved GSH synthesis may involve increased efflux of cystein with the increase of GSH synthesis which mediated by the secretion of soluble growth factors by MSCs or by the interaction of MSCs with host cells or both. In this study MSCs implantation significantly increased SOD and PON-1 in renal tissue. These findings clearly illustrated the antioxidant efficacy of MSCs in attenuating the oxidative damage of LPS and confirmed the results of Gorbunov *et al* [42] and Quintanilha *et al* [43]. Furthermore the obtained results elicited the efficient role of kefir in the potentiation the antioxidant effect of MSCs.

Inflammation is the first response to infection and is critical to body defense, macrophages are regarded as a major component in the inflammatory response [44-46]. The large amounts of inflammatory cytokines can activate and secrete by LPS and macrophages stimulation leading to cascade of inflammatory response [47]. To characterize the anti-inflammatory mechanism of kefir and / or MSCs in LPS-induced renal damage, the present study focused on the measurement of serum levels of TNF- α , IL-10 as well as mRNA expression of NF- κ B and iNOS in renal tissue.

The obtained results revealed that, the sole administration of kefir or MSCs implantation significantly increased the serum level of IL-10, decreased TNF- α in association with significant decrease in mRNA expression of NF- κ B and iNOS. The obtained reduction in TNF- α and increase IL-10 level in serum demonstrate that, this alteration occurs on a systemic level and proved the efficiency of the examined probiotic and MSCs in the improvement of immunity function through the stimulation of IL-10 production with the inhibition of pro-inflammatory cytokine, TNF- α . This beneficial effect demonstrates the efficacy of the examined material in shifting the balance of cytokine away from the pro-inflammatory cytokine towards the production of anti-inflammation ones. The recorded increase in serum IL-10 of kefir treated group is in the harmony with the finding of Vinderola *et al* [48] who reported that kefir microflora induced the production of IL-10 producing cells. Meanwhile, the modulating immunity function of MSCs could be referred to their interaction with circulating and tissue monocytes and macrophages to reduce the harm that caused by unbridled immune response to host tissue with increasing the production of IL-10 and decreasing TNF.

The nuclear factor kappa B (NF- κ B) is a pivotal regulator of pro-inflammatory gene expression and is thought to be associated with some inflammatory disease [49,45]. During sepsis activation of NF- κ B leads to an increase in cytokines and chemokines expression which cause organ injury. Blocking NF- κ B could be a strategy to protect against LPS-induced organ damage [2]. In this study the recorded decrease in mRNA expression of NF- κ B through kefir and / or MSCs treatment reflects the potential effect of the examined material. This finding runs parallel with Ma *et al* [50] who reported a direct decrease in pro-inflammatory cytokines via down-regulation of NF- κ B activity using probiotics. It was also reported

that kefir probiotic could inhibit the innate response of intestinal epithelial cells, suggesting the modulation of NF- κ B pathway [51]. Results of the current study demonstrate that MSCs transplantation significantly alleviate the experimentally induced AKI and protecting renal tissue. This protection includes inflammatory response, oxidative stress and tissue repair that proved the ability of MSCs to evoke the endogenous repair mechanism in the kidney. It has been reported that renal inflammation has been illustrated to be modulated by alteration of the status of infiltrating cells by changing the status of activation or stimulating regulatory cells [52]. This is particularly the case in stem cell therapy due to its potential in regeneration of renal tissue [53].

Renal dysfunction and apoptosis is importance for the pathophysiological consequence of AKI. Apoptosis is a crucial event that initiates inflammation and subsequent tissue damage [54]. Apoptosis is judged in this work by the measurement of mRNA expression of caspase-3 in renal tissue. The recorded increase in caspase-3 expression suggests that apoptosis plays a significant role in LPS – induced AKI. Kefir administration significantly attenuated the mRNA expression of caspase-3, this finding could be attributed to the increased production of anti-inflammatory IL-10 [55]. The recorded down-regulation of caspase-3 in the renal tissue reflects the protective role of kefir against apoptosis and supports its antioxidant properties. This finding agrees with Kanbak *et al* [20], who reported the deactivated effect of kefir on caspase-3 expression. Treatment with kefir and /or MSCs, reduced caspase-3 expression and caused improvement in rat kidney function compared to those of LPS challenge group at the under taking time. These results confirm the efficacy of MSCs in inhibiting the inflammatory response and renal cells apoptosis to repair the damaged renal tissue. This finding agrees with the work of Shin *et al* [25].

Data of the present study elicited the additional effect of pre- and post-administration of kefir probiotic in reducing LPS-induced oxidative stress in renal tissue through the attenuation of free radical generation, this finding is the harmony with previous that proved the protective effect of kefir on spinal cord ischemic / reperfusion [56], and liver dysfunction [57].

5. Conclusion

In conclusion, our results revealed that, the toxic effect of LPS on renal tissue is continued and progressed through the experimental period. Administration of kefir and /or MSCs successful battle and attenuated this toxicity through the one and seven days after LPS injection. The obtained data clearly proved the protective effect of kefir through its antioxidant and anti-inflammatory effects. The results also proved that the combination of the probiotic with MSCs markedly enhanced the efficiency of stem cells therapeutic action through the potentiation of anti-inflammatory and immune-modulation response of MSCs with attenuation the underlying pathological process which results in progression of kidney disorder. Our results revealed that, the combination of kefir probiotic with stem cell therapy could be of interest in clinical trials towards AKI model and further study with longer follow up would be necessary.

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