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Research Article

THE KEY ROLE OF KEFIR PROBIOTIC IN ENHANCEMENT THE THERAPEUTIC ACTION OF MESENCHYMAL STEM CELLS IN LPS- INDUCED LIVER INJURY

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ABSTRACT

Acute liver injury occurs after intraperitoneal injection of lipopolysaccharide (LPS). The release of pro-inflammatory cytokines and oxidative stress are both important in the pathogenesis of LPS-induced liver injury. This study investigated the key role of kefir probiotic in enhancement therapeutic actionof mesenchymal stem cell (MSCs) against LPS-induced liver injury.Sixty female rats were divided into 6 groups (10 rats each),Control group, LPS treated group, LPS + probiotic, probiotic + LPS + probiotic, LPS + MSCs and probiotic + LPS + MSCs +propiotic. Hepatic function, cytokine level, MDA, GSH and the activity of antioxidant enzymes SOD and PON1 as well as the m RNA expression of NF-κB, iNOS and caspase-3 were monitored in rat's liver. Injection of LPS significantly increased ALT, AST, TNF-α in association with dramatic decrease of serum albumin and IL-10 levels. Oxidative stress evidenced by increased MDA in association witha significant decline of GSH content and the antioxidant enzymatic activity of SOD and PON1. Furthermore, theRNA expression of NF-κB, iNOS and caspase-3 were significantly up-regulated in liver tissue. Administration of kefir or MSCs alone significantly suppressed LPS-induced liver injury. The pre and co-treatment of kefir with MSCs potentiate the therapeutic action of MSCs. Pre and co-treatment of kefir probiotic with mesenchymal stem cell transplantation could be viable approach for the treatment of liver injury.

Keywords: Endotoxin, Liver oxidative stress, inflammatory response, Probiotics and mesenchymal stem cells

INTRODUCTION

Lipopolysaccharides (LPS) are glycolipids found in abundance on the outer membrane of gram-negative bacteria and have the ability to incite a vigorous of inflammatory response. In humans monograms of LPS injected into the blood stream can result in all the manifestation of septic shock, hepatotoxicity and multiple organ failure **[1, 2, 3]**.

Endotoxin is a potent activator of liver macrophages and endothelial cells, stimulating these cells to produce inflammatory and cytotoxic mediators including TNF-α, reactive oxygen and nitrogen species which have been implicated in hepatotoxicity with the failure of host defense mechanism to block the pathogen invasion which leads to microbial proliferation with the release of microbial products that activate the host inflammatory responses **[4]**.

Several methods including the usage of antibiotics, prebiotics and probiotics can be used to prevent the overgrowth of pathogen. Compared to prebiotic and antibiotic therapy, probiotics strains are safer and less expensive therapy. Kefir is one of the well-known probiotics, that are originally defined as “microorganisms causing growth of other microorganism” and later on as live microorganism which when consumed in adequate amounts result in health benefits of the host **[5]**.

The probiotics could influence the gut-liver axis; these microorganisms have also been proposed a possible adjunctive therapy in some types of liver diseases **[6]**. Preliminary data obtained in rat models of alcoholic and nonalcoholic steatohepatitis showed that the treatment with Probiotics could be effective in limiting liver damage **[7, 8, 9]**.

Bone marrow mesenchymal stem cells are pluripotent stem cells with the potential to differentiate into liver cells**[10, 11]**.Recent studies have also shown that Bone marrow mesenchymal stem cell play a substantial role in liver fibrosis treatment without allograft rejection.A number of

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clinical trials also proved that Bone marrow mesenchymal stem cellcan effectively alleviate end stage liver disease and improve symptoms and liver function indicating the effectiveness and safety of Bone marrow mesenchymal stem cell in clinical implantation**[12, 13, 14]**.However it has also been reported that Bone marrow mesenchymal stem cells have the potential to promote fibrosis in liver tissue **[15, 16]**.Recently neutral stem cell therapy and concomitant of antioxidant have shown to cause significant therapeutic role in liver and lung **[17, 18]**. Although previous studies showed that probiotics have protective role in different types of liver injury and fibrosis **[6, 19]**, their role with mesenchymal stem cells (MSCs) transplantation in liver injury remains uncertain. Consequently more investigations are still to be required. Therefore this study is preformed to investigate the effect of probiotics or MSCson LPS- included liver injury, in addition to prove or disprove the role of kefir probiotic in enhancement of MSCstherapeutic action.

1. Materials and Methods

1.1. 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 femalewhite 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 resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO_2 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 resuspended in serumsupplemented medium and incubated in 50 cm^2 culture flasks (Falcon). The resulting cultures were referred to as first-passage cultures **[20]**.Cells were identified as being MSCs by their morphology, adherence and their power to differentiate into osteocytes **[21]** and chondrocytes **[22]**.

1.2. Preparation of kefir

Kefir was purchased from organic culture (Buckly,SUSA).20 mg freeze-dried kefir grains was added to 100 ml of pasteurized milk and fermentation was carried out at 23˚Cfor 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 **[23]**.

2.3. Animals and Experimental design

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:

Group 1: Control normal group

Group 2: LPS, the rats of this group were injected intraperitoneally with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS (pH 7.4) to induce endotoxemia**[24]**. LPS (extracted from *Escherichia Coli* 0111:B4) was purchased from Sigma-Aldrich (St Louis, MO).

Group 3: LPS + kefir, the rats of this group were injected intraperitoneally with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, then 30 minutes after, and administration of kefir was carried out for 7 days by gavage, at a dose of 1.8 ml / day for each rat **[23]**.

Group 4: kefir +LPS +kefir, the rats of this group were injected intraperitoneally with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS and treated with kefir that administration for 7 days before the LPS injection and was maintained for other 7 days.

Group 5: LPS + MSCs, the rats of this group were injected intraperitoneally with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, then rats were received MSCs , which were processed and cultured for 14 days, in a dose 3×10^6/ rat **[20]** as a single dose by intravenous infusion (IV) at the rat tail vein 30 minutes after LPS injection.

Group 6: kefir+ LPS + MSCs + kefir, the rats of this group were injected intraperitoneally with 10 mg / kg b.wt. of LPS in 1 ml of sterile PBS, and treated with a dose of MSCs (3×10^6/ rat **[20]**as a single dose) by IV infusion at the rat tail vein 30 minutes after LPS injection, rats of this group also treated with kefir administration for 7 days before the LPS injection and was maintained for other 7 days after MSCs infusion.

All samples in all groups were collected after 7 days of MSCs infusion. Blood samples were collected from the retro orbital vein. Sera were separated and used for the measurement of alanine aminotransferase

(ALT) and aspartate aminotransferase (AST), Albumin (ALB), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10). Fresh liver tissue were removed from the decapitated rats for the determination ofmalondialdehyde **(**MDA), reduced glutathione (GSH), superoxide dismutase (SOD) andparaoxonase-1(PON-1) as well as the quantitative analysis ofnuclear factor kappa B(NFκB),inducible nitric oxide synthase **(**iNOS) and caspase-3 gene expression by Real Time PCR.

2.4. Biochemical Analysis

2.4.1. Measurement of liver functions:

Serum ALT, AST and ALB were detected by conventional laboratory investigation.

2.4.2. Measurement of hepatic MDA level:

To measure the MDA concentration **[25]**, 100 mg of liver tissue was homogenizedin 1 mL PBS, pH 7.0, with micro pestle in micro tube. 20 % TCA was added to liver homogenate to precipitate the protein, and centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. The MDA concentration was calculated using 1,1,3,3, tetraethoxypropane as standard.

2.4. 3. Measurement of hepatic GSH content:

GSH concentrationwas measured from liver homogenate in phosphate buffer pH 8.0 and then 5% TCA was added, to precipitate liver protein. After centrifugation, 5, 5-dithiobisnitrobenzic acid (DTNB) solution was added to the supernatants of liver homogenate, and incubated for1hour. The absorbance was measured.Concentration of GSH in liver tissue was calculated using reduced glutathione standard curve **[26]**. Liver protein concentration was calculated by using standard curve of bovine serum albumin (BSA) solution.

2.4.4. Measurement of hepatic SOD activity:

SOD activity in liver homogenate was measured through the inhibition of nitroblue tetrazolium (NBT) reduction by O_2-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 **[27]**.

2.4.5. Measurement of paraoxonase-1(PON-1) activity:

Tissues PON-1 enzyme activity: PON-1 activity towards paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate) was determined by measuring the initial rate of substrate hydrolysis to p- nitrophenol, whose absorbance was monitored at 405 nm in the assay mixture, A PON1 activity of 1 U/mg protein was defined as 1 µmol p-nitrophenol formed per minute per mg protein **[28]**.

2.4.6. Estimation of serum levels of IL-10 and TNF-α:

Serum IL-10 and TNF-α were assayed by commercially available Enzyme-linked immunosorbent assays (ELISA) kitssupplied by Q &D system Quatin USAaccording to the manufacturer`s instructions.

2. 5. Quantitative RT PCRforNFκB,iNOS and caspase-3 gene expression in liver tissue:

Total RNA was extracted from liver tissue homogenate usingRNeasypurification reagent (Qiagen, Valencia, CA). cDNA was generated from 5 µg of total RNA extracted with 1 µl (20 pmol) antisense primer and 0.8 µl 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 GenBank (Table 1). All primer sets had a calculated annealing temperature of 60°. Quantitative RT-PCR was performed in duplicate in a 25-µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2–3 µl of cDNA. Amplification conditions were 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 s 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 NFκB mRNA was calculated using the comparative threshold cycle method (Ct). All values were normalized to the β-actin gene **[29]**.

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

Gene	Primer sequences
NFκB	Forward primer: 5'-GCTTACGGTGGGATTGCATT-3' Reverse primer: 5'-TTATGGTGCCATGGGTGATG-3'
Caspase-3	Forward primer:ATGGACAACAACGAAACCTC Reverse primer: TTAGTGATAAAAGTACAGTTCTT
Inos	Forward 5' GCAGGATCAGAACACAGCAA3' Reverse 5' ATGGTTACGGGAGGGGTAAG 3'
β-actin	Forward primer :5'CCAGGCTGGATTGCAGTT3' Reverse primer: 5'GATCACGAGGTCAGGAGATG3'

2.6. Statistical Analysis

The results were expressed as the mean ± standard deviation (SD) for six animals in each group. One-way analysis of variance (ANOVA) was used to compare group variables. The pairwise comparisons were conducted using Mann-Whitney U test. Differences between groups were assessed by one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant. Data were statistically analyzed using the statistical package for social science 16 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Effect of kefir and / orMSCs on LPS included liver function:

To confirm the effect of kefir, MSCs or their combination in protecting rats from hepatic damage, serum ALT, AST and ALB were examined, Table 2 shows that, injection of LPS caused a marked rise in serum levels of ALT and AST activities. This increase in the examined hepatic marker enzymes is associated with a significant reduction of ALB level when statistically compared with the control non- treated group. In contrast, it was found that, the 7 days administration of kefir or MSCs alone after LPS injection significantly improved the induced hepatic damage (Table 2). The obtained results revealed that, the administration of kefir for 7 days before and after LPSchallenge augmented the obtained improve in the examined liver function tests and a more pronounced effect was obtained in the group of rats administered kefir for 7 days before LPS challenge andtreated with MSCsin concomitant with kefir administration for other 7 days (Table 2).

Table 2: Effect of kefir probiotic and mesenchymal stem cells on serum levels of liver enzymes and albumin in LPS – injured rats

Parameters / Groups	ALT (U / L)	AST (U / L)	ALB (g / dL)
G1: Control	22.05 ± 2.79 ^{a}	20.12 ± 0.69 ^{a}	4.57 ± 0.53 ^{e}
G2: LPS	80.62 ± 2.72 ^{e}	79.02 ± 4.18 ^{f}	1.53 ± 0.31 ^{a}
G3: LPS + Kefir	50.42 ± 2.87 ^{d}	47.17 ± 6.59 ^{e}	2.75 ± 0.31 ^{b}
G4: kefir +LPS +Kefir	39.88 ± 2.08 ^{c}	38.97 ± 2.38 ^{d}	3.37 ± 0.37 ^{c}
G5: LPS + MSCs	33.67 ± 2.91 ^{b}	34.65 ± 2.76 ^{c}	2.91 ± 0.17 ^{b}
G6:Kefir+LPS+MSCs+Kefir	30.75 ± 6.40 ^{b}	25.87 ± 4.19 ^{b}	4.14 ± 0.17 ^{d}

Values are mean ± SD (n= 6 rats). In the same column, mean values with different superscript symbol(s) are significantly different ($p< 0.05$).

3.2. Effect of kefir and/or MSCs on LPS-induced oxidative stress:

Challenging with LPS induced a marked increase in hepatic MDA level when measured after 7 days of LPS injection (Table 3). This increment of hepatic MDA level is associated with asignificant decrease in hepatic GSH content. The treatment of LPS challenged rats with kefir or MSCs alone produced a significant attenuation in LPS induced oxidative stress. The obtained results also revealed that, the 7 days pre and post administrationof kefir not only proved the antioxidant effect of kefir probiotic but

also proved its protective role against the generation of oxidative stress. The obtained results also revealed that, the transplantation of MSCs attenuated the oxidative stress induced by LPS challenge and this attenuating effect was potentially enhanced by the pre and co-administration of kefir with MSCs in LPS challenged rats (Table 3).

3.3. Effect of kefir and/ orMSCs on hepatic SOD and PON1 activities:

Hepatic SOD and PON1 significantly reduced in LPS challenged rats when compared with control non-treated group (Table 3). Kefir or MSCs administration significantly increased the activities of SOD and PON1 when statically compared with LPS treated group. Pre and co-administration of kefir with MSCs to LPS injected rats not only assessed the antioxidant and the protective effect of kefir probiotic but also proved the enhanced effect of kefir to potential the therapeutic effect of MSCs (Table 3).

Table 3: Effect of kefir probiotic and mesenchymal stem cells on hepatic MDA and GSH levels as well as SOD and PON1 activity in LPS – injured rats

Parameters / Groups	MDA (nM/mg protein)	GSH (nM/mg protein)	SOD (U/mg protein)	PON1 (U/mg protein)
G1: Control	1.38 ± 0.20 ^{a}	53.92 ± 5.62^{d}	2.43 ± 0.32^{f}	123.92 ± 9.86^{d}
G2: LPS	17.12 ± 2.64^{e}	14.58 ± 2.08 ^{a}	0.31 ± 0.05 ^{a}	46.67 ± 6.36^{a}
G3: LPS + Kefir	6.54 ± 1.04^{d}	35.43 ± 3.43^{b}	0.89 ± 0.11^{b}	84.85 ± 8.38 b
G4: kefir +LPS +Kefir	3.51 ± 0.39^{c}	47.4 ± 3.59 ^{c}	1.67 ± 0.17^{d}	99.67 ± 3.07 ^{c}
G5: LPS + MSCs	6.88 ± 0.84^{d}	33.37 ±3.13^{b}	1.19 ± 0.23^{c}	94.72 ± 10.25^{c}
G6:Kefir+LPS+MSCs+Kefir	2.6 ± 0.31^{b}	48.97 ± 5.79^{c}	2.07 ± 0.17^{e}	121.9 ± 5.18 ^{d}

Values are mean ± SD (n= 6 rats). In the same column, mean values with different superscript symbol(s) are significantly different (p< 0.05).

3.4. Effect of kefir and/orMSCs on circulating TNF-α and IL-10 in LPS-challenged rats:

Serum levels of TNF-α were significantly elevated in all groups over the normal control non treated rats (Table 4). In all treated groups, rats exhibited a significant reduction in serum TNF-α compared to LPS-injected group. Pre and post treatment ofLPS-challenged ratswith kefir or MSCsalone showed more significant reductions in serum TNF- α compared to rats treated with kefir alone. Further significant reduction in TNF-α was observed in kefir pre and in combined treatment with MSCs relative to other treated groups. On the other hand, serum levels of the anti- inflammatory cytokine IL-10 were significantly decreased in the treated groups. All treatment regimens caused significant elevations in serum levels of IL-10 with the highest observed increase in the group of rats' pre and post treated with kefir and MSCs (Table 4).

Table 4: Effect of kefir probiotic and mesenchymal stem cells on serum levels of TNF-α and IL-10 in LPS – injured rats

Parameters / Groups	TNF-α (pg / ml)	IL-10 (pg / ml)
G1: Control	30.25 ± 4.13^{a}	135.38 ± 9.75^{d}
G2: LPS	137.37 ± 10.73^{f}	29.03 ± 3.86^{a}
G3: LPS + Kefir	98.6 ± 8.57^{e}	88.25 ± 3.47^{b}
G4: kefir +LPS +Kefir	64.95 ± 10.14^{c}	112.02 ± 7.15^{c}
G5: LPS + MSCs	79.22 ± 9.48^{d}	111.55 ± 7.41^{c}
G6:Kefir+LPS+MSCs+Kefir	53.22 ± 8.63^{b}	128.23 ±7.24^{d}

Values are mean ± SD (n= 6 rats). In the same column, mean values with different superscript symbol(s) are significantly different (p< 0.05).

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3.5. Kefir and/ orMSCs attenuated LPS induced, NFκB activation, iNOS as well as caspase-3 in rat's liver:

Real time quantitative PCR was used to quantify the levels of NFκB, iNOS, and caspase-3 mRNA in liver tissue, figures 1, 2 and 3, revealed that NFκB, iNOS and caspase-3 mRNA were significantly lower in kefir or MSCstreated groups than LPS group after 7 days of LPS challenge. A more pronounced effect was observed in the groups of rats, pre and post treated with kefir and MSCs.

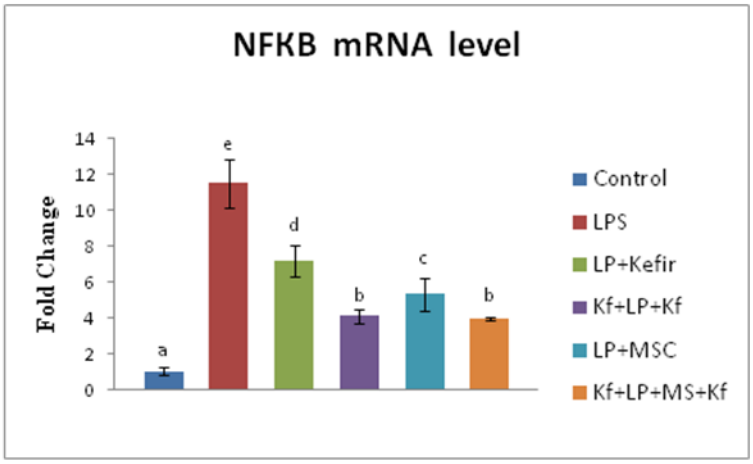

Figure 1: Expression level of NFKB gene in the different groups. The steady-state levels of mRNA in the liver were analyzed by real-time PCR assay. Values are presented as means ± SD (n=6). β- Actin was used as an invariant internal control for calculating mRNA-fold changes. Mean values with different superscripts are significantly different ($p < 0.05$).

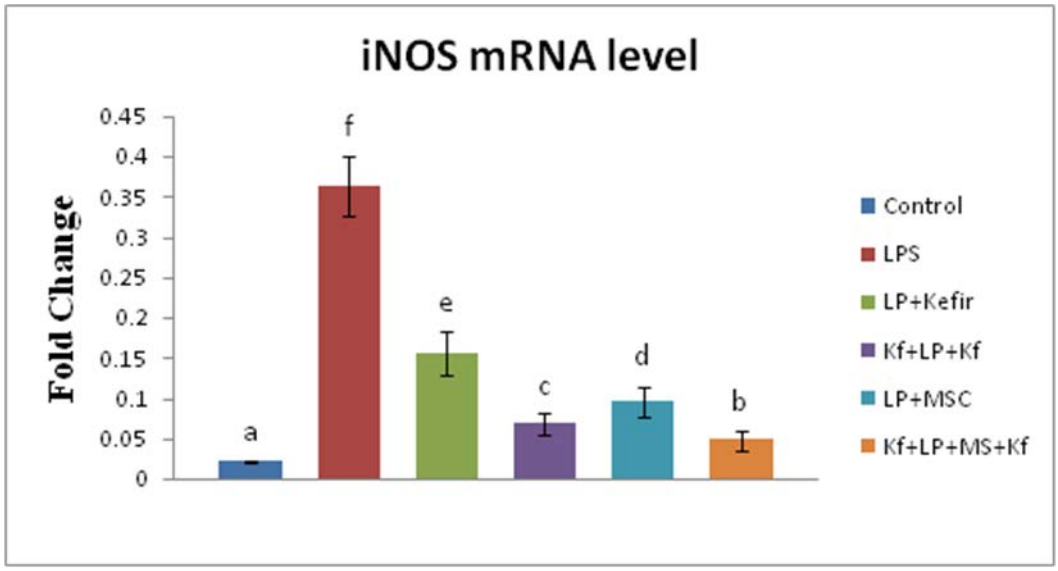

Figure 2: Expression level of iNOS gene in the different groups. The steady-state levels of mRNA in the liver were analyzed by real-time PCR assay. Values are presented as means ± SD (n=6). β- Actin was used as an invariant internal control for calculating mRNA-fold changes. Mean values with different superscripts are significantly different ($p < 0.05$).

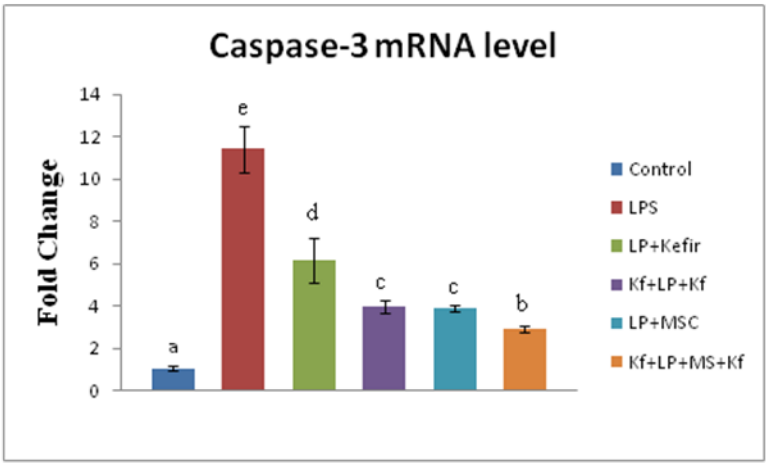

Figure 3: Expression level of Caspase- gene in the different groups. The steady-state levels of mRNA in the liver were analyzed by real-time PCR assay. Values are presented as means ± SD (n=6). β- Actin was used as an invariant internal control for calculating mRNA-fold changes. Mean values with different superscripts are significantly different ($p< 0.05$).

4. Discussion

In this study we found that LPS significantly inducehepatic injury and decrease the capability of liver cells to produce protein as evidenced by the obtained increase of aminotransferases activity and the reduction of albumin level. This finding is in consistent with several studies that documented the role of LPS in the induction of liver damage **[30,31]**. In this study, the experimental condition evoked the mechanism involved in liver injury in particular the oxidative stress that evidenced through the increased concentration of hepatic MDA in association with decreased of GSH content as well as the antioxidant enzyme activities namely, SOD and PON-1. The obtained inhibition of SOD activity is referred to the implication of superoxide anion ($O_2^{\cdot-}$) as one of the toxic mediators responsible for the most toxicities observed in LPS-induced cellular injury **[3]**. PON-1 is a calcium-dependent esterase synthesized primarily in the liver and it has been revealed that PON-1 is an antioxidant enzyme and also protects HDL and LDL from oxidation **[32]**. In the present study, it was observed that injection of LPS to rats caused a significant decrease in hepatic PON-1 activity. This decrease may be attributed to the direct toxic effect of LPS on hepatocytes or the increased free radical production.

In this study, 7 days of kefir administration after LPS injection improved the functional activity of the liver and increased serum albumin level. This finding might reflect the protective role of kefir in the protection of liver tissue. In addition the administration of kefir either pre or post LPS challenge (group 3 and 4, Table 2) significantly attenuated the oxidative damage of LPS as indicated by the significant decrease of hepatic MDA level in association with enhance the antioxidant status of liver tissue. These findings proved the efficiency of kefir probiotic in preserving the cell membrane integrity of hepatic cells and support the results of Liu *et al.* **[33]** who attributed the antioxidant activity of kefir to its proton – donating power and SOD like activity. Moreover, Güven*et al*.**[19]** compared the anti-oxidative consequence of kefir and vitamin E against oxidative damage of CCl_4 in animal model and reported that, both kefir and vitamin E have the capacity to protect tissue against CCl_4-induced damage, and kefir offer more protection as compared to vitamin E.

Data of the present study confirmed that animals in the cell treated groups (G5 and G6, Table 2) showed better results for the biochemical parameters. The serum liver injury markers (ALT, AST) were reduced pronouncedly with MSCs infusion, suggesting protection of hepatocytes from necrosis and

apoptosis because ALT and AST are enzymes that reveal hepatocytes damage. The obtained pronounced decrease in these enzymes with the increase of Albumin level strongly support the hepatic protective effect of MSCs against LPS-induced liver injury through stabilizing and maintaining the hepatocyte membrane, as well as repair damaged hepatic tissue by stimulating hepatocyte regeneration and hepatocyte proteinsynthesis as described by Zhao*et al.***[34].**

In the present study, MSCs transplantation showed anti-peroxidative effect in liver tissue by significantly decreasing LPS-induced rise of liver MDA. This effect of MSCs may contribute to the regional suppression of the activated macrophages and endothelial cells as confirmed by the pronounced decrease of ALT and AST activities.MSCs significantly increased the hepatic content of GSH. This finding is consistent with that of Iyer*et al*. **[35]**who reported that, the potential mechanism by which MSCs improves GSH contents may involve increased efflux of cystein with the increase of GSH synthesis which mediated by the secretion of soluble growth factors by MSCsor by the interaction of MSCs with host cells or both. Furthermore MSCs infusion is associated with increase of SOD and PON-1 activities in liver tissue. This finding indicates that, MSCs significantly decreased the oxidative injury. This study clearly demonstrates the anti-oxidative efficacy of MSCs in combating the induced oxidative damage of LPS and confirmed the results of Gorbunov*et al.* **[36]** andQuintanilha*et al.* **[37]** who reported the antioxidant efficiency of MSCs against LPS and thioacetamide –induced liver oxidative damage in animal models. Furthermore theobtained results elicited the efficient role of kefir administration in enhancement the antioxidant effect of MSCs treatment.

To characterize the anti-inflammatory mechanism of kefir and MSCs in LPS induced liver damaged, the present investigation have been focused on the measurement of serum levels of TNF-α, IL-10 as well asmRNA expression of NFκB, and iNOS in liver tissue. It was found that the sole administration ofkefir or MSCs transplantation significantly increased the serum level of IL-10, decreased TNF-α level in association with down regulation of NFκB, and iNOS in liver tissue. The obtained decrease of TNF-α and increase IL-10 level in serum demonstrate that, this alteration occurs on a systemic level and proved the efficiency of the test materials in the improvement of immunity function through the stimulation of IL-10 production with the inhibition of pro-inflammatory cytokine. Therefore, the observed beneficial effect could be due shifting the balance of cytokine away from the pro-inflammatory cytokine towards the production of anti- inflammatory ones. The recorded increase in serum IL-10 in kefir treated groups runs parallel with the finding of Vinderola*et al*. **[38]** who reported that kefir microflora induced the production of IL-10 producing cells among adherent cells derived from Peyer's patches of mice. Meanwhile, the modulating immunity function of MSCs could be referred to their interaction with circulating and tissue monocytes and macrophages and reprogram them with the increased production of IL-10 in association with decreased circulation of TNF-α which reduces harm caused by unbridled immune responses to host tissues as describe by Németh*et al.***[39]**.

It has been reported that blocking NFκB could be a strategy to protect against endotoxemia **[40]**. The recorded down regulation of NFκB through kefir and MSCs administration reflect the efficiency of the test materials in controlling the production of inflammatory cytokine through the modulation of NFκB and iNOS mRNA expression in addition to the obtained decrease in the serum level of TNF- α. This finding runs parallel with the finding of Ma *et al*. **[41]**who reported a direct decrease in pro-inflammatory cytokines via the down regulation of NFκB activity using probiotics, moreover Ulisse*et al.,***[42]** reported that the anti-inflammatory action of the probiotic treatment appears to involve the inhibition of NFκB activation by blocking κB-α ubiquitination and degradation. Results of the present study demonstrate that, MSCs transplantation significantly alleviate the experimentally induced liver injury and protecting the liver tissue. This protection is multi-factorial including inflammatory response, oxidative stress and tissue repair that proved the efficiency of MSCs to evoke the endogenous repair mechanism in the liver. It has been reported that MSCs are capable of producing a variety of cytokines and hematopoieticsoluble growth factors**[43-45]**. Among the great number of growth factors liver protective effects have been attributed to hepatocyte growth factor (HGF). Wang *et al*. **[46]**

reported that bone marrow derived mesenchymal cells (BMSCs) protected hepatic stellate cells through paracrine signaling by HGF secreted by BMSCs that leads to NFκB inhibition.

Traditionally, hepatocyte apoptosis is considered as a characteristic feature of acute liver injury, and increased evidence indicates hepatocytes apoptosis plays a dominant role in the pathogenesis of hepatic failure as well **[47]**. Apoptosis is judged in this work by mRNA expression level of cspase-3 measurement in liver tissue. The recorded increase in caspase-3 expression suggests that, apoptosis plays a significant role in LPS- induced liver damage and support the finding of Schäfer*et al*. **[48]** who reported thatcaspase-3 is one of the effectors caspases downstream of apoptotic pathways and is recruited especially when apoptosis is triggering by extrinsic signals as seems to happen in LPS.

Data of the present study significantly support the anti-inflammatory and anti-oxidative role of kefir which is responsible in the preventative effect of the early onset of LPS-induced liver damage.In addition Kefir administration significantly attenuated the mRNA expression of caspase-3 in liver tissue, this finding could be attributed to the increased production of IL-10 and support the finding of Zhong*et al*. **[49]** who reported that, IL-10 protect liver tissue by direct down regulation of apoptotic cytokine secretion and indirectly by counteracting the pro-apoptotic action of the pro-inflammatory cytokine. The down regulation of caspase-3 in liver tissue reflects the protective role of kefir against apoptosis and indicating its promising effect on LPS-induced apoptosis. This finding supports the antioxidant role of kefir in suppressed the apoptosis in liver tissue and suggests that ROS generated by LPS likely play a critical triggering role in apoptotic cell death in LPS-induced liver damage.

At 7 daysof MSCs treatment, the liver cell apoptosis was significantly reduced compared with those of LPS challenge group and rat liver function significantly improved. These results confirm that MSCstransplantation can inhibit inflammatory response and liver cell apoptosis to repair the damaged liver tissue and MSCs are of certain therapeutic effect for liver injured rats. This finding is in accordance with the observation of Yuan *et al.* **[50]** who reported that, after liver cell transplantation, early immune rejection showed transplanted liver cell apoptosis, which is one of the important factors affecting the liver cell transplantation and under normal circumstances, the liver cells will gradually go through apoptosis three days after transplantation, but acute liver failure patients need at least 5 to 7 days to recover liver function. Therefore, reduction of the occurrence of apoptosis in cell transplantation is the key to the effectiveness of cell transplantation. The decrease in mRNA expression of caspase-3 and the significant recovery in rat liver function that was observed after MSCs transplantation, suggests that one of the mechanism of MSCs in the treatment of liver injury is that the reduction of caspase-3 expression.

The results of the present study demonstrate that Kefir or MSCs alone significantly suppressed LPS-induced inflammation, oxidative stress and apoptosis, meanwhile, MSCs transplantation displayed a more pronounced effect in attenuating the induced reduction of liver function. The obtained results elicited the additional protective effect of pre and post treatment of Kefir to reduce the oxidative stress through the attenuation of free radical generation. Consequently pre and co treatment of Kefir with MSCs potentiate the therapeutic effect of MSCs and illustrates a significant beneficial effects over the MSCs therapy alone.

In summary, the present study provides evidence that oral administered of Kefir probiotic enhances the therapeutic effect of mesenchymal stem cells against LPS induced liver injury in rats via attenuating liver oxidative damage, pro-inflammatory cytokine production, suppressing NFκB expression with increasing IL-10 production. These findings indicate that kefir in combination with BMSCs can function as a potent immunomodulator that preventing the underlying pathological process which results in progressing of liver damage. Because kefir in combination with BMSCs has been found to protect rats against LPS- induced model of liver oxidative damage, a combination of kefir and BMSCs may be of interest for clinical use.

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