Pro-oxidant and degenerative effects of haloperidol under inflammatory conditions in rat; the involvement of SIRT1 and NF-κB signaling pathways

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Article Info

Abstract

The present study was conducted to evaluate the effects of different doses of haloperidol (HP) on induction of oxidative stress in blood and liver cell degeneration in comparison with influences of HP pre-treatment on inflammatory process induced by intraperitoneal (IP) administration of lipopolysaccharide (LPS). One hundred twenty male albino Wistar rats were randomly divided into eight groups (15 in each), including: Control group, LPS group, three groups as HP administration in three divided doses (0.50, 1.00 and 2.00 mg kg^{-1}), and three treatment groups that HP was administered in three doses (0.50, 1.00 and 2.00 mg kg^{-1}) prior to LPS administration. Concentrations of malondialdehyde, activities of antioxidant enzymes including glutathione peroxidase, superoxide dismutase and also the levels of tumor necrosis factor-alpha and interleukin 1-beta were measured in blood and serum. In addition to liver histopathological changes evaluation, hepatic silent information regulator of transcription 1 (SIRT1) and phosphorylated-nuclear factor-κB (p-NF-κB) levels were quantitated. Our findings indicated that sole administration of HP (particularly higher doses) can induce oxidative stress in blood and cell degeneration in liver, while it can attenuate inflammatory process induced by LPS administration presumably via SIRT1 up-regulation and preventing the induction of p-NF-κB. The oxidative and degenerative effects of HP and its impact on inflammatory status were completely dose-dependent according to our results. The possible anti-inflammatory effects of HP may affect reparative mechanisms and hepatic cell degeneration. However, the influences of HP on immune system need further investigations and its higher doses should be administered cautiously especially in patients with immune system dysfunctions.

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Introduction

Haloperidol (HP), a widely used neuroleptic drug for treatment of acute and chronic psychosis, schizophrenia and Tourette syndrome has been shown to induce oxidative stress.[1,2] Centrally, HP is a dopamine-receptor antagonist, therefore, elevated levels of dopamine in the synaptic clefts increase the activity of mono-amine oxidases leading to increased generation of reactive oxygen species (ROS).[2,3] Peripherally, HP is metabolized by the liver cytochrome enzymes and the activation of these enzymes can generate free radicals and induce oxidative stress in the liver.[4] Recent studies have shown that HP increases malondialdehyde (MDA) production in the liver and serum.[5,6] Long term administration of HP could induce cell degeneration in the livers of rats with high doses inducing more intense cell damage and degenerative changes.[7] Studies also have stated that HP can be cytotoxic *in vitro* [8,9] and also *in vivo*.[10] It has recently been shown that HP could dose-dependently suppress NF-κB signaling pathway, which mediates immune responses and inflammation, as well as the production of pro-inflammatory cytokines by the macrophages.[11,12] It has also been reported that HP administration could down-regulate production of pro-inflammatory cytokines via blocking peripheral dopamine receptors, the effect which significantly aids in the management of rheumatoid arthritis.[13] In addition, another study indicated that HP could inhibit LPS-induced release of interleukin-8 from peripheral monocytes while other antipsychotic drugs

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showed no such effects.[14] Another groups of studies reported that HP administration for seven days prior to inflammation induction by carrageenan injection could boost inflammatory effects of carrageenan in rat[15] and also pre-treatment with HP 0.10 mg kg^{-1} in rat could intensify primary inflammatory response and suppress secondary inflammatory response after chronic auto-immune inflammation induction by Freund's adjuvant.[16] Interestingly, such ambiguous behavior about dopaminergic cytotoxic and cytoprotective effects have been reported by several studies and it has been demonstrated that dopamine can behave as either an antioxidant or pro-oxidant molecule in different concentrations.[17,18] In addition, it has been reported that dopamine together with catecholamines can affects lymphoid organs and immune cells function through sympathoadernergic terminals.[19]

Lipopolysaccharide (LPS), a bacterial antigen, is a powerful activator of macrophages. The activation of toll-like receptor 4 (TLR4) by LPS in macrophages leads to the activation of NF-κB and increased production of pro-inflammatory cytokines like TNF-α and IL-1β, a cascade that result in the production of ROS by the phagocytic cells i.e. macrophages and neutrophils.[20,21] Additionally, LPS induces hepatic cell injuries followed by inflammation and the infiltration of the inflammatory cells into the liver tissues.[22,23]

Silent information regulator of transcription 1 (SIRT1) stimulates Forkhead box O1 (FoxO1) that in turn up-regulates the expressions of the antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and thereby aids in resisting against oxidative stress and reducing ROS levels.[24,25] An antagonistic cross-talk exists between SIRT1 and NF-κB as SIRT1 up-regulation suppresses NF-κB activities, highlighting the possible contribution of SIRT1 activators to the treatment of inflammatory disorders.[26-28]

As it has been mentioned above while some studies reported that HP could potentially induce oxidative stress in the brain, liver, and serum and also it can be cytotoxic, other studies have expressed that HP could attenuate the production of pro-inflammatory cytokines and the activities of NF-κB with resultant anti-inflammatory effects. Despite accumulating *in vitro* evidence, the literature is poor regarding the *in vivo* effects of HP on the serum oxidative stress status and the levels of the inflammatory mediators. Therefore, the aim of the present study was to examine serum MDA, GPx, superoxide dismutase (SOD) as well as tumor necrosis factor-alpha (TNF-α) and IL-1β levels with and without the induction of inflammation via LPS and simultaneously to evaluate hepatic SIRT1 and phosphorylated-NF-κB levels in the rats since liver is the main organ involved in the metabolism of psychotropic agents including HP.[29] We assumed that there may be an association between oxidative stress

induction by different doses of HP and their possible inhibitory effects on proinflammatory cytokines through the involvement of SIRT1 pathway and its antagonistic cross-talk with NF-κB.

Materials and Methods

Drugs and chemicals. HP and LPS were purchased from Sigma Co. (St. Louis, USA). Ethanol 99.00% (as HP solvent) was purchased from Merck (Darmstadt, Germany).

Animals. One hundred twenty male albino Wistar rats, 3-month-old and 190 - 200 g weight were purchased from the Pasteur Institute of Iran (Amol, Iran). Animals had free access to water and food under a 12:12 hr light-dark cycles, 25.00 ℃ ambient temperature with 70.00% humidity. All animal experiments were performed under protocols approved by the ethical committee of the Urmia University. All procedures of this study were carried out in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Veterinary Medicine, Urmia University (Protocol No: IR-UU-AEC-210/AD/3).

Experimental procedure. Animals were divided into eight groups (each n = 15): Control, LPS (1.00 mg kg^{-1}), HP (0.50 mg kg^{-1}), HP (1.00 mg kg^{-1}), HP (2.00 mg kg^{-1}), HP (0.50 mg kg^{-1}) + LPS (1.00 mg kg^{-1}), HP (1.00 mg kg^{-1}) + LPS (1.00 mg kg^{-1}) and HP (2.00 mg kg^{-1}) + LPS (1.00 mg kg^{-1}). The calculated dose of HP was dissolved in 250 μL ethanol and then diluted with water. HP was administered through intra-gastric gavage for eight consecutive days on a daily basis. Plasma peak concentrations of HP (oral and muscular injection) in human are achieved on the 6th day after consecutive daily administrations[30,31] and an approximate similar pattern is seen in the rats.[32] Rats body weight were recorded daily for exact dose calculation. In the control group the mixture of 250 μL ethanol and water were used through gavage. LPS was dissolved in normal saline and injected 1.00 mg kg^{-1} IP. According to previous studies LPS has been used from 0.10 μg kg^{-1} up to 1000 μg kg^{-1} to induce inflammation.[33,34]

Blood and liver tissue sampling. After eight days of HP administration, LPS and normal saline were injected intraperitoneally and blood samples were taken from the tail vein 30 min, 3 and 6 hr after LPS administration, in control and three HP group saline normal was administered instead of LPS. For this purpose, 15 rats in each group were subdivided into three subgroups of 5 animals each. Serum/Plasma was prepared by centrifugation at 1,500 *g* for 5 min and then stored at – 70.00 ℃. Liver samples were extracted from the animals euthanized by carbon dioxide (CO_2) on the 8th day of the study after each blood sampling time (30 min, 3 and 6 hr after LPS IP injection).

Biochemical and immunochemical tests. The plasma samples were assayed for MDA levels HPLC system (Agilent 2000, Agilent Technologies, Santa Clara, USA).

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The activities of the antioxidant enzymes including SOD (Randox, Dublin, Ireland) and GPx (Randox) were measured in blood. Moreover, serum samples were assayed for TNF-α (Rat TNF alpha ELISA Kit; Abcam Cambridge, USA) and IL-1β (Rat IL-1beta ELISA Kit; Abcam) according to the manufacturer's instructions.

Histopathological evaluation. Liver samples were fixed in 10 % neutral buffered formalin. Fixed liver tissues were embedded in paraffin, cut into 5.00 μm for each sample, stained with Hematoxylin and Eosin and examined under light microscope. The evaluation of liver sections was performed on midzonal regions of liver lobule based on the severity of histopathological changes (degeneration, cell infiltration, and hemorrhages) according to following scores: 0: None, 1: Mild, 2: Moderate and 3: Severe.[35]

Western blotting. Fifty mg of liver tissues were lysed in radio-immuno-precipitation assay (RIPA) buffer (SantaCruz, Dallas, USA) and the total protein concentrations of liver tissues were measured by Lowry method. For p-NF-κB measurement, first nuclear and cytoplasmic fractions were isolated by nuclear extraction kit (Cayman Chemical, Ann Arbor, USA). Then, protein separation was done by SDS-PAGE and the obtained bands were transferred onto the PVDF membranes via electro-blotting. The membranes were incubated in NF-κB (sc-8008; SantaCruz), p-NF-κB (sc-136548; SantaCruz), SIRT1 (sc-74465; SantaCruz), and β-actin (sc-47778; SantaCruz) primary antibody solutions at 4.00 ℃ overnight after a blocking step with nonfat milk solution for 60 min. Next, the membranes were incubated in the horse radish peroxidase conjugated secondary antibody solutions at room temperature for 45 min. Finally, the blots were visualized using a Pierce ECL Western Blotting substrate (Thermo Fisher Scientific, Waltham, USA). On the pictures obtained from x-ray films, band densities were quantitated with the Image J. software (version 1.41; National Institutes of Health, Bethesda, USA). The SIRT was measured in the total cell lysate and β-actin was used as the loading control for these measurements, by contrast, p-NF-κB was assayed in the nuclear fractions with total NF-κB being as the loading control. The obtained pixel intensities for each group were first divided by the intensities of their respective loading controls and then the calculated values for study groups were normalized by values obtained for the control group.

Statistical analysis. All statistical analyses were carried out using SPSS Software (version 19.0; IBM, Armonk, USA). Two-way analysis of variance (ANOVA) was performed in a repeated measure design followed by LSD post hoc test. All experiments were performed in triplicates and data were expressed as Mean ± SD. Differences were considered significant at $p < 0.05$.

Results

Biochemical and immunochemical parameters. As seen in Figure 1A, a significant increase in plasma MDA levels could be seen in LPS, HP 0.50 mg kg⁻¹, HP 1.00 mg kg⁻¹, HP 2.00 mg kg⁻¹, HP 0.50 mg kg⁻¹ + LPS, HP 1.00 mg kg⁻¹ + LPS, and HP 2.00 mg kg⁻¹ + LPS groups. The LPS receiving groups demonstrated a time-dependent increase in their plasma MDA levels. Figure 1 shows that either HP (in all three doses) or LPS can induce oxidative stress in the blood, elevate MDA levels and the activities of SOD and GPx. MDA levels were significantly reduced in HP 1.00 mg kg⁻¹ + LPS and HP 2.00 mg kg⁻¹ + LPS groups and had a significant difference with LPS group. As shown in Figures 1B and 1C, SOD and GPx activities were significantly increased in all groups in comparison with the control group 6 hr after LPS and normal saline IP injection. There was also a significant difference between LPS group with 1.00 mg kg⁻¹ HP+LPS and 2.00 mg kg⁻¹ HP+LPS groups. Despite, the activity of SOD was significantly higher than 1.00 mg kg⁻¹ HP+LPS and 2.00 mg kg⁻¹ HP+LPS groups, and the activity of GPx was significantly higher in 1.00 mg kg⁻¹ HP+LPS and 2.00 mg kg⁻¹ HP + LPS groups in comparison with LPS group. According to Figure 1D, there was no significant difference between TNF-α levels in HP 0.50 mg kg⁻¹, HP 1.00 mg kg⁻¹ and HP 2.00 mg kg⁻¹ groups with control. However, TNF-α level was significantly increased in LPS group 30 min after LPS IP injection in comparison with 0.50 mg kg⁻¹ HP+LPS, 1.00 mg kg⁻¹ HP+LPS, and 2.00 mg kg⁻¹ HP+LPS groups. Pre-treatment with HP significantly decreased TNF-α level 30 min after LPS IP injection. There was also no significant difference between 3 and 6 hr after LPS administration between TNF-α levels in LPS, 0.50 mg kg⁻¹ HP+LPS, 1.00 mg kg⁻¹ HP+LPS, and 2.00 mg kg⁻¹ HP+LPS groups. As demonstrated in Figure 1E, there was no significant difference between IL-1β levels in HP 0.50 mg kg⁻¹, HP 1.00 mg kg⁻¹ and HP 2.00 mg kg⁻¹ groups compared to the control group, while a remarkable increase in the level of IL-1β was observed in LPS group in comparison with 0.50 mg kg⁻¹ HP+LPS, 1.00 mg kg⁻¹ HP+LPS, and 2.00 mg kg⁻¹ HP+LPS groups. IL-1β levels were higher 6 hr after LPS injection in LPS and HP0.5+LPS groups indicating the time dependent increase in IL-1β levels after LPS administration. There was no significant difference in IL-1β levels between LPS group and HP0.5+LPS group. However, in HP1+LPS and HP2+LPS groups IL-1β levels was significantly decreased 6 hr after LPS IP injection in comparison with LPS group, regarding pre-treatment with HP.

Liver SIRT1 and p-NF-κB levels. As seen in Figure 2A and 2B, p-NF-κB amount was elevated in LPS group and there was a significant decrease in p-NF-κB levels in HP0.5+LPS and HP2+LPS in comparison with LPS group. Besides, p-NF-κB amounts were significantly lower in HP2+LPS in comparison with HP0.5+LPS.
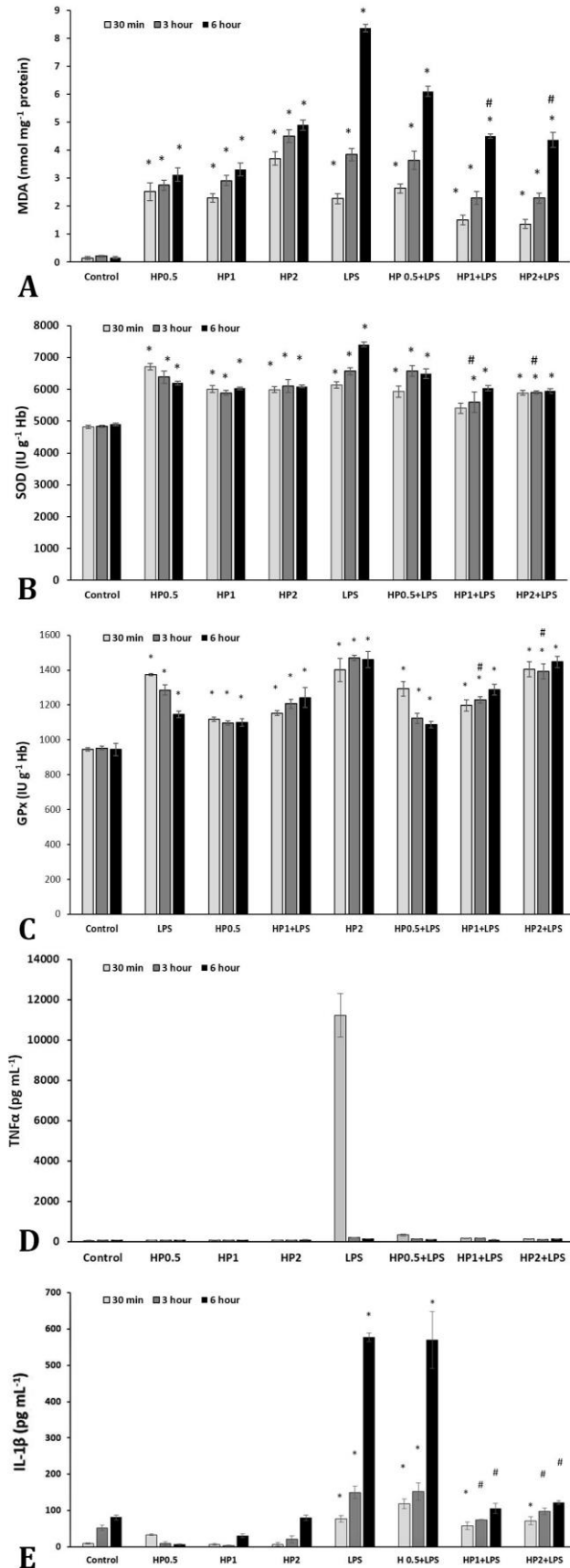
Fig. 1. Effects of LPS, HP, and combined HP and LPS on **A)** plasma levels of MDA, **B)** activities of SOD and **C)** GPx, **D)** levels of TNF-α and **E)** IL-1β (proinflammatory cytokines) in rat's serum after eight days gavage and 30 min, 3, and 6 hr after LPS and normal saline IP injection. Control, healthy control rats; HP (0.5), 0.50 mg kg^{-1} HP; HP (1), 1.00 mg kg^{-1} HP; HP (2), 2.00 mg kg^{-1} HP; LPS, rats receiving only LPS injection; HP (0.5) + LPS, 0.50 mg kg^{-1} HP plus LPS IP injection after eight days of gavage; HP (1) + LPS, 1.00 mg kg^{-1} HP plus LPS IP injection after eight days of gavage; HP (2) + LPS, 2.00 mg kg^{-1} HP plus LPS IP injection after eight days of gavage. All experiments were performed in triplicates and data were presented as mean ± SD. * indicates significant difference with control group at $p < 0.05$, and # indicates significant difference with LPS group at $p < 0.05$.

According to Figure 2A and 2C SIRT1 levels were dose-dependently increased by HP administration. There was a significant difference between SIRT1 levels in LPS group in comparison with HP0.5, HP2, HP0.5+LPS and HP2+LPS groups and pre-treatment with HP dose dependently elevated SIRT1 levels. Moreover, SIRT1 amounts were significantly higher in HP2+LPS in comparison with HP0.5+LPS.

Histopathological findings. The liver architecture of the control group was normal with intact hepatocytes and portal areas throughout the liver tissue (Figs. 3A and 4). In LPS group, severe inflammatory cell infiltration along with hyperemia and degenerative processes, hydropic and fatty degeneration, and necrosis of hepatocytes were observed in liver tissue sections in this group (Figs. 3B and 4). Similar histological findings to control group were seen in 0.50 mg kg^{-1} HP rats, except for a mild inflammatory cell infiltration and slight edema around the portal area (Fig. 3C and Fig. 4). In 1.00 mg kg^{-1} HP treated rats, normal hepatic cellular architecture was distorted due to mild to moderate infiltration of immune cells and small foci of necrosis were observed (Figs. 3D and 4). HP (2.00 mg kg^{-1}), heavy inflammatory cell infiltration in portal areas (also throughout the liver), severe hyperemia with noticeable degeneration and very extensive necrosis in the hepatocytes were seen. Histological examination showed significant differences in comparison with the control group ($p < 0.01$), (Figs. 3E and 4). In, HP 0.50 mg kg^{-1} + LPS, severe inflammatory cell infiltration and hyperemia were observed similar to LPS group ($p < 0.05$). On the other hand, degenerative processes (vacuolar degeneration) were throughout the liver tissue sections, however, their severity were not as much as the LPS treated group 6 hr after LPS administration ($p < 0.05$), (Figs. 3F and 4). HP 1.00 mg kg^{-1} + LPS, moderate alterations of hepatic tissue was observed (Figs. 3G and 4). The HP 2.00 mg kg^{-1} + LPS, surprisingly showed less severe inflammatory alterations in contrast to only LPS treated group ($p < 0.05$), (Figs. 3H and 4).
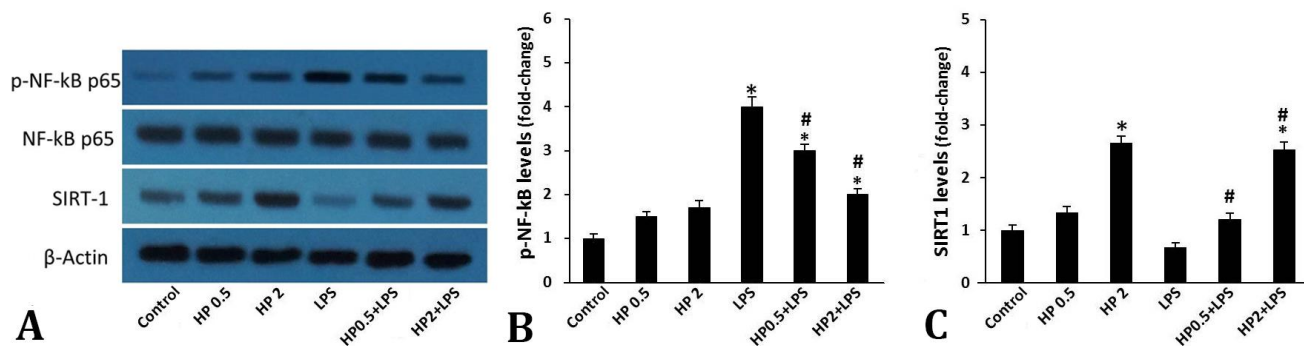
Fig. 2. Effects of LPS, HP, and combined HP and LPS on hepatic phosphorylated NF-κB levels (A and B) and hepatic SIRT1 levels (A and C) in rats after eight days gavage and 6 hr after LPS and normal saline IP injection. Control, healthy control rats; HP (0.5), 0.50 mg kg^{-1} HP; HP (2), 2.00 mg kg^{-1} HP; LPS, rats receiving only LPS injection; HP (0.5) + LPS, 0.50 mg kg^{-1} HP plus LPS IP injection after eight days of gavage; HP (2) + LPS, 2.00 mg kg^{-1} HP plus LPS IP injection after eight days of gavage. **A)** Representative bands of western blotting. **B** and **C)** Semi-quantitative analysis was performed using the image J software, intensities of the correspondent SIRT1 bands were normalized by β-actin values and then related to normal control rats. In case of phosphorylated-NF-κB, the total NF-κB values were used as the loading control and normalization calculations. All experiments were performed in triplicates and data were presented as mean ± SD. * indicates significant difference from control group at $p < 0.05$ and # indicates significant difference from LPS group at $p < 0.05$.
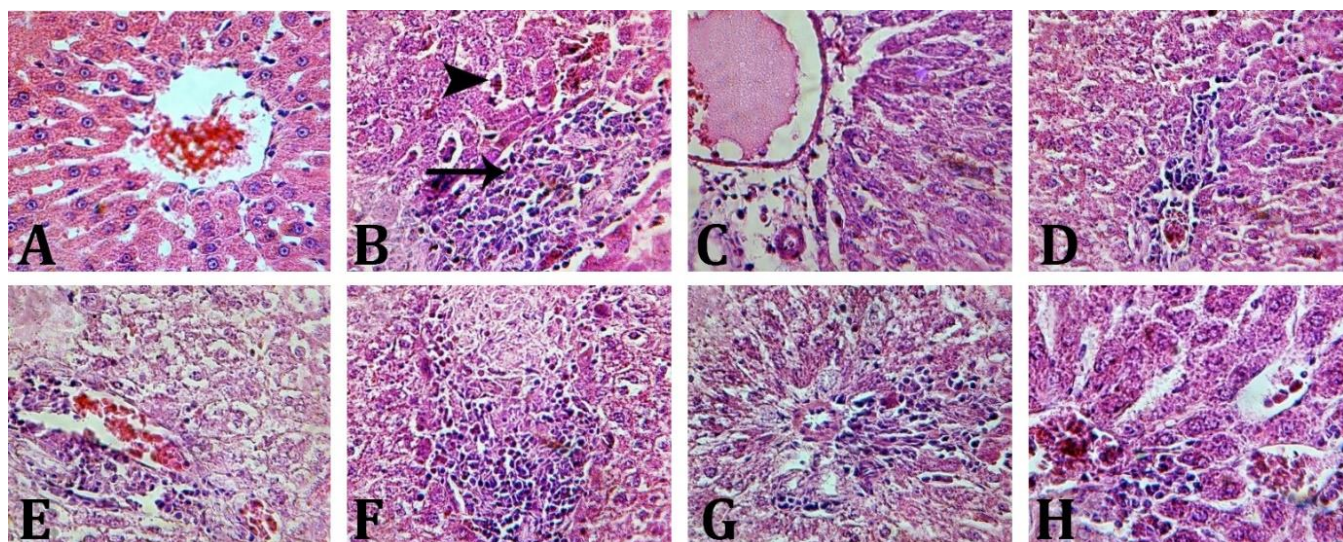

Fig. 3. Micrographs of liver tissue showing effects of haloperidol and LPS alone and their co-treatment on inflammatory and degenerative processes of liver. **A)** Control group, normal architecture of liver tissue is seen, (H & E, 400×). **B)** LPS group, histopathological changes: Inflammatory cell infiltration (long arrow), sinusoidal dilation and hepatocyte degeneration and necrosis (arrow head) are seen, (H & E, 200×). **C)** Haloperidol low dose, mild histopathological changes are seen, (H & E, 400×). **D)** Haloperidol medium dose, moderate histopathological changes are seen, (H & E, 200×). **E)** Haloperidol high dose, extensive histopathological alteration as severe inflammatory cell infiltration and necrosis of hepatocytes are seen, (H & E, 400×). **F)** Haloperidol low dose + LPS, shows severe and notable histopathological changes, (H & E, 200×). **G)** Haloperidol medium dose + LPS, moderate to severe histopathological changes are seen. Haloperidol high dose + LPS, moderate histopathological changes are seen, (H & E, 400×).

Discussion

Despite numerous studies on HP and its effects on the pro-inflammatory cytokines and induction of oxidative stress, the literature is poor regarding the effects of different doses of HP on inflammation status and oxidative stress. In addition, studies are controversial about either inflammatory or anti-inflammatory effects of HP. While some studies have reported that HP could affect inflammatory process by preventing the release of cytokines followed by NF-κB suppression or peripheral

dopamine receptors blockade,[11-13] others indicated that HP could intensify oxidative stress and increase ROS production and also it could boost inflammation.[15,36] Moreover, the effects of different doses of HP on the oxidative stress, proinflammatory cytokines (TNF-α & IL-1β) and liver histopathological alterations under inflammatory conditions and their associations remains to be elucidated. In the present study, we considered blood samples as a general panel among all tissues and organs for MDA, antioxidant enzymes and proinflammatory cytokines evaluation and also
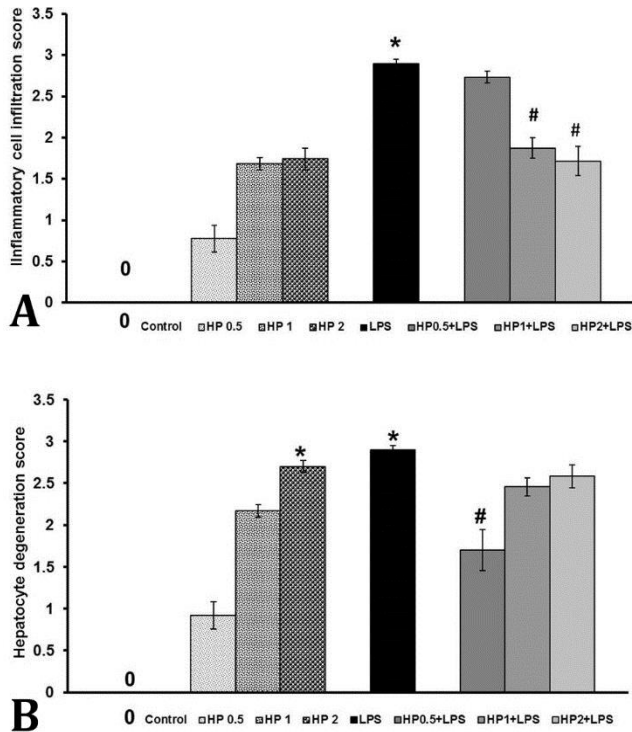
Fig. 4. Effects of HP and its post-treatment with LPS on **A)** inflammatory cell infiltration and **B)** hepatocyte degeneration in liver tissue of rat. Data are presented as mean ± SEM (n = 8). * indicates significant difference from control group at $p < 0.05$ and # indicates significant difference from LPS group at $p < 0.05$.

we adopted liver to investigate its histopathological alterations, SIRT1 and p-NF-κB levels as liver is one of the main influenced organs after HP administration.[29] Our findings showed that HP could elevate MDA levels and induce oxidative stress in blood in all three doses (0.50, 1.00 and 2.00 mg kg^{-1}) and it could cause noticeable cell degenerations in the liver only at the highest dose. It seemed that the higher doses of HP induced more intense oxidative stress and therefore higher MDA levels and reparative increase in antioxidant enzymes (SOD & GPx) activity were seen in comparison with the control group.

Previous studies showed that HP increased MDA production in liver and serum[5,6] that was in agreement with our findings and as seen by histopathological results, HP derived hepatic cells to swelling and degeneration in a dose-dependent manner. Halici *et al.* reported that long-term HP induced cell degeneration in rat livers with higher doses having more degenerative effects.[7] The findings which were in agreement with our observations. Severe cell degeneration and inflammatory cell infiltration in LPS group were obvious which could acknowledge previous studies.[37] Furthermore, according to our results the amounts of SIRT1 in liver tissue were elevated especially with higher doses of HP which could explain the increased activity of SOD and GPx in blood. Our findings also showed

that there were no significant effects on p-NF-κB in liver tissue as well as TNF-α and IL-1β levels in serum by HP alone in comparison with control group. In fact, our study indicated that higher doses of HP could suppress NF-κB presumably by SIRT1 overexpression which was in agreement with studies reporting the inhibitory effects of SIRT1 on NF-κB expression.[26-28] Moreover, cell degeneration observed followed by higher doses of HP could be related to NF-κB suppression. According to previous studies the suppression of NF-κB induces cell apoptosis, degeneration and suppresses proliferation.[38,39]

It has been shown that LPS could induce oxidative stress via activating toll-like receptor 4 (TLR4) on the macrophages triggering NF-κB over-activation and increased production of pro-inflammatory cytokines, this cascade, finally, resulted in the activation of phagocytic cells and generation of ROS.[20,21] Moreover, the toxic effects of LPS are mediated by endogenous cytokines and the release of other pro-inflammatory mediators via LPS-activated macrophages.[40-44]

Elevations of pro-inflammatory cytokines to their peak levels 2 hr after LPS injection and 3 hr after LPS oral administration induce neutrophil death which result in enhanced oxidative stress after 3 hr.[45,46] Earlier studies has shown that 2 to 6 hr was the best time for pro-inflammatory cytokines induction and the peak of LPS effects would occur in 6 hr.[47] In addition, TNF-α expression was one of the earliest events following by inflammatory response initiation. It has been reported that TNF-α could intercede many crucial incidents upon both acute and chronic inflammatory process initiation including: Adhesion molecules manifestation and up-regulation of other primitive cytokines like IL-1 and IL-6.[48]

In our study LPS injection increased MDA levels, antioxidant enzymes activities in blood in a time-dependent manner, especially 6 hr after LPS injection which could acknowledge the previous studies. As shown by our results, enhanced oxidative stress after 3 hr up to 6 hr and severe inflammatory cell infiltration along with degenerative processes were observed in liver tissue sections 6 hr after LPS administration. Our findings also were in agreement with previous studies on TFN-α and IL-1β secretion after LPS administration and as seen by our results there was an immediate increase in TNF-α level 30 min after LPS IP injection. As been reported by previous studies, the elevated amounts of TNF-α followed by LPS administration could result in IL-1 and IL-6 production and as shown by our results there was a crucial increase in TNF-α level 30 min after LPS administration leading to IL-1β enhancement 3 and 6 hr after LPS IP injection in LPS group. Also previous studies showed that LPS administration resulted in hepatic inflammation and cell injuries in liver [22,23] which were consistent with our study 6 hr after LPS administration.

Interestingly, according to our results, there was a significant enhancement in p-NF-κB amounts in liver tissues 6 hr after LPS administration in comparison with control group which could justify the elevated amounts of TNF-α and IL-1β in blood.[20,21] In addition, as seen by our findings there were no significant changes in amounts of SIRT1 in LPS group in comparison with control group which was in agreement with previous studies on antagonistic cross-talk between SIRT1 and NF-κB.[26-28] It seems that increased activities of antioxidant enzymes in blood as seen by our results was not associated with SIRT1 up-regulation by LPS in 6 hr and it might be due to previously existed antioxidant enzymes in the blood and if we were supposed to consider more evaluation times after 6 hr we would see different results.

As observed in our findings there was a dose-dependent reduction in MDA, TNF-α and IL-1β levels in blood and also p-NF-κB amounts in liver especially 6 hr after LPS injection in comparison with the LPS group in receiving HP groups for eight days prior to LPS IP injection, suggesting that higher doses of HP could prevent LPS-induced p-NF-κB, proinflammatory cytokines, and also inflammatory cell infiltration in HP 1.00 mg kg^{-1}+ LPS and HP 2.00 mg kg^{-1} + LPS groups in comparison with LPS group. Previous studies showed that HP could suppress NF-κB and proinflammatory cytokines release by macro-phages in a dose-dependent manner.[11,12] However HP 0.50 mg kg^{-1} did not attenuate LPS-induced inflammatory process generally. According to our finding low doses of HP had significant inhibitory effects on hepatic cells degeneration induced by LPS. The fact that HP higher doses could prevent LPS based inflammatory responses in our study was presumably by the involvement of SIRT1 and NF-κB antagonistic cross-talk disclosing the ambiguous behavior of HP and this was somehow in agreement with both controversial groups of previous studies clarifying the dose dependent different behaviors of HP.

As outlined above, it seems that the oxidative and degenerative effects of HP and its impact on inflammatory status is completely dose dependent. Regarding the general effects of HP on induction of oxidative stress, cell degeneration and inflammatory status in comparison with LPS group, there is an obvious fact indicating that HP can have oxidative and degenerative effects, however, it may also attenuate inflammatory process at the same time.

According to clinical trials on the treatment of chronic and acute psychosis and agitation it has been reported that the administration dosage of HP may exceed 35.00 mg intravenously, especially in the management of acute psychosis, and in some cases over 100 mg.[49] The HP involved signaling and mechanisms interfering degenerative process along with inhibitory effects on inflammatory parameters should be considered especially in chronic liver diseases and also patients with immune system dysfunctions.

Most recently a newly found HP derivative, *N*-n-Butyl haloperidol iodide, has been shown to activate SIRT1/FoxO1/Mn-SOD antioxidant signaling and protect myocardial hypoxia/reoxygenation injury,[50] representing the idea which HP and its derivatives can be a highly recommended issue for researchers in the field especially immunology. The possible effects of HP on cytokines and immune responses should be considered even clinically, particularly in high dose administration.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interests regarding the publication of this article, financial and/or otherwise.

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