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# Protective Effect of *Lycium Barbarum* extract on Lipopolysaccharides and/or Gamma-Irradiation – Induced Hepatorenal Toxicity in Rats

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## ABSTRACT

In this investigation, the protective effect of *Lycium barbarum* extract (LBE) on lipopolysaccharides (LPS) and/or gamma-irradiation (IRR) – induced hepatorenal toxicity in rats was evaluated. Rats intoxicated with LPS or IRR showed alterations in activities of ALT, AST, ALP and GGT, as well as levels of triglycerides, total cholesterol, urea, creatinine, albumin, and total protein content, hematological indices, oxidative stress markers (malondialdehyde, nitric oxide, reduced glutathione), antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), and inflammatory markers (Tumor Necrosis Factor-alpha (TNF-α), Interleukins (IL-1β, IL-2, IL-6), Nuclear Factor-kappa B (NF-κB) and prostaglandin E2 (PGE2) level). Also, upregulation of the gene expressions of inducible nitric oxide synthase (iNOS), nuclear-factor - kappa B p65 subunit (NF-κB p65), cyclooxygenase 2 (COX-2), toll-like receptor 4 (TLR4) and myeloid differentiation factor 88 (MyD88) in the liver and kidney tissues were observed. The level of calcium was raised in the hepatic and renal tissues of LPS, and IRR intoxicated groups. However, the combined treatment (LPS/IRR) enhanced the examined oxidative stress, and inflammation in liver and kidney tissues. LBE administration demonstrated regulation of the observed oxidative stress and inflammation induced in the hepatic and renal tissues of LPS, IRR and LPS/IRR intoxicated rats. Conclusion: The results demonstrated that LBE has protective effects by preserving the liver and kidney tissues from the oxidative stress and the inflammation that triggered by LPS, IRR and their combined toxicity, which is mediated by regulation of calcium level and COX-2/PGE2/TLR4/MyD88/NF-κB pathway.

***Keywords:*** *Lycium barbarum* extract, lipopolysaccharides (LPS), gamma-irradiation, hematology, hepatorenal toxicity, TLR4; MyD88, rats.

## 1. Introduction

Lipopolysaccharides (LPS) or endotoxins are lipophilic components of the outer cell membrane of Gram-negative bacteria. LPS stimulates the production of numerous inflammatory cytokines, such as TNF-α, and IL-6, speeds up the NF-κB motivation, triggers TLR4 pathways, and enhances apoptosis. This inflammatory response to LPS appears locally, as well as systematically, in a process comparable to the LPS prompted humans' septic shock (Tjahjono *et al*., 2021). LPS are the ideal templates to explore the systemic inflammation in the experimental animals (Cheng *et al*., 2017). LPS toxicity accompanied by histological and biochemical changes in liver and kidney tissues, which can be progressed into cancer (Beyoğlu and Idle, 2013; Huang *et al*., 2013; and Rousta *et al*., 2018).

On the other hand, ionizing-radiation (IR) has a variety of advantageous manipulations in medication including diagnosis and therapy of diverse tumors, and malignant diseases (Boerma and Hauer-Jensen, 2011). Radiation-related disorders are a current health problem with broad-spectrum medical of social and economic consequences (Said *et al.,* 2012). All types of ionizing radiation generate ions that form reactive oxygen species (ROS) and other radicals due to the oxidative stress. These ROS attack various cellular macromolecules such as DNA, lipids, and proteins that leads to cell

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death, thus restrict the efficiency of the radiotherapy (Esposito *et al.,* 2014). Exposures to environmental hazards, including ionizing radiation, have prompted investigations of natural substances that protect human health (Said *et al.,* 2012)

Remediation using plant-based agents as nutraceuticals are considered the most efficient in the management of free radical related pathological conditions (Kalra, 2003). Numerous studies focused on the protective effects of natural compounds or plant extracts on various liver and kidney injuries *in-vivo* (Tu *et al.,* 2015). Since the polyphenolic-compounds have strong antioxidant properties, they can protect against oxidative stress (Alkhalf and Khalifa, 2018). *Lycium barbarum* (LB) *L* fruits (Goji berries or *Fructus lycii*) are traditional medical herbs of the *Solanaceae* plant family. LBE is used in numerous industries that utilized as a nutritional and food accompaniment (Masci *et al.,* 2018). LBE demonstrates plentiful pharmacological and biochemical activities due to its various phytochemicals' constituents, such as polysaccharides, polyphenols, fatty acids, and vitamins (Zhou *et al.,* 2020). The LBE has a restricted protection to liver, kidney, lung, heart, and brain organs as a result of antioxidant, immunomodulation, anti-inflammatory, anti-carcinoid effect and some other health-supporting events (Cheng *et al.,* 2015 and Kulczyński and Gramza-Michałowska, 2016). This study was aimed to evaluate the protective effects of LBE against LPS/IRR-induced hepatorenal toxicity in rats.

## 2. Materials and methods

### 2.1. Chemicals

Lipopolysaccharides (LPS) (from Escherichia Coli 0111:B4, EC number: 297-473-0) and the other reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). *Lycium barbarum* Fruits were purchased from local market.

### 2.2. Preparations of *Lycium barbarum* water extract (LBE)

To prepare LB extract (LBE), the dried parts of the Lycium fruits (50.0 g) were placed in 1000 mL of distilled water and then extracted by heating for 3 h 115 °C. After extraction, the solution was filtered out, and let to dry (Oh *et al*., 2012).

In a preliminary experiment, six animals per group were administered orally with different concentrations of LBE (50, 100, 200, 500, 1000 mg/kg.b.wt.), which revealed non-toxic-symptoms for the next twenty-four hours. Rats were administered orally with LBE (100 mg/kg body weight (kg. b. wt)/day) five days per week, for 4 weeks. No toxic symptoms were observed during this treatment period in animals treated only with LBE.

### 2.3. Irradiation operation

The Whole-body gamma-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), using $^{137}$Cesium Canadian Gamma Cell-40. The radiation dose rate was 0.666 rad/sec at the time of exposure. The total radiation dose was 8 Gy (2 Gy every week for 4 weeks) as fractioned doses of the whole-body.

### 2.4. Animals

Wistar male albino rats (weighing 200-250 g) were obtained from the breeding unit of the NCRRT. The animals were housed according to the standard sanitary conditions of suitable temperature, humidity, and illumination states. They were freely allowed to standard pellet diet (21% protein) and fresh water ad-libitum at the NCRRT animal residence, and habituated for one week. This investigation was achieved by conferring the experimental animals' international strategies and permitted by the NCRRT-Ethical Committee (24A/21).

### 2.5. Experimental design

Rats were randomly divided into eight groups (12 animals/group):

Group 1 (Control; C): rats treated orally with 1.0 mL of tap water, and 0.2 ml of saline intraperitoneally (IP).

Group 2: rats treated with 1.0 mL of LBE (100 mg/kg. b. wt), orally) five days per week, for 4 weeks.

Group 3: rats treated with LPS (0.25 mg/kg. b. wt, dissolved in saline, IP) five days/week, for 4 weeks (Imajo *et al*., 2012).
Group 4: Gamma-irradiation treated rats (2 Gy every week for 4 weeks).
Group 5: rats treated with0.2 mL of LPS (0.25 mg/kg. b. wt, dissolved in saline, IP) five days/week (Imajo *et al*., 2012), and exposed to gamma-irradiation (2 Gy/week) for 4 weeks,
Group 6: rats treated orally with 1.0 mL of LB extract (100 mg/ kg. b. wt) then treated with 0.2 mL of LPS (0.25 mg/kg. b. wt, dissolved in saline, IP) five days per week, for 4 weeks.
Group 7: rats treated with 1.0 mL LB extract (100 mg/ kg. b. wt, orally) five days per week, then exposed to gamma-irradiation (2 Gy/week) for 4 weeks.
Group 8: rats treated orally with 1.0 mL of LB extract (100 mg/kg. b. wt), then injected IP with 0.2 mL of LPS (0.25 mg/kg. b. wt, dissolved in saline) five days per week (Imajo *et al*., 2012), and exposed to gamma-irradiation (2 Gy/week) for 4 weeks.

At the end of the four weeks, after an overnight fasting period, all animals were euthanized under urethane anesthesia (1.2 g urethane/kg. b. wt (Moheban *et al*., 2016)) by cervical dislocation. Blood was assembled, stood to coagulate at 37 °C for 30 minutes, then, centrifuged at 1200 g, at room temperature, for 15 min using a universal centrifuge (16R, Germany) to separate the blood serum and preserved at -80 °C for the biochemical analysis and inflammatory markers assessments. The liver and kidneys tissues were dipped in ice-cooled 0.9% NaCl physiological solution, dried, and preserved at -80 °C.

**2.5. Biochemical assessments:**

Blood biochemical parameters (ALT (Alanine Amino-Transaminase), AST (Aspartate Amino-Transferase), ALP (Alkaline Phosphatase), GGT (Gamma-Glutamyl Transferase), TC (total cholesterol), TG (triglycerides), HDL-Chol (high density lipoprotein-cholesterol), urea, creatinine (Creat), ALB (albumin), and TP (total protein)) were determined on Alfa Wassermann Diagnostic Technologies, LLC, ACE, Alera (USA) biochemical blood analyzer, at the Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt. However, the serum of lactate dehydrogenase (LDH) activity was assessed using the MyBioSource rat Lactate Dehydrogenase (LDH, Cat No. MBS269777) ELISA kit.

**2.6. Hematologic indices assessments**

The automated Hematology-Analyzer (XT-2000i, Sysmex Corporation, KOBE, JAPAN) at the Regional Center for Food and Feed (RCFF), Agriculture Research Center, Giza, Egypt, the hematologic indices in the EDTA-blood samples were determined.

**2.7. Preparation of liver and kidney homogenates**

The liver or kidney tissue samples were homogenized 10% (w/v, by ice-cold 0.15 M KCl solution, at pH 7.4), then centrifuged at 1200 g for 15 min at 4 °C, to prepare the liver, and kidney tissues' homogenates, as described previously (Eassawy *et al.,* 2021 and Salem and Ismail, 2021).

**2.8. Assessment of the oxidative stress markers**

Malondialdehyde (MDA) (Satoh, 1978), nitric oxide (NO) (Montgomery and Dymock, 1961), the reduced glutathione (GSH) (Ellman, 1959), superoxide dismutase (SOD) (Nishikimi, *et al.,* 1972), catalase (CAT) (Aebi, 1974), glutathione peroxidase (GPX) (Paglia, and Valentine, 1967), and glutathione reductase (GR) (Goldberg *et al.,* 1983) activities, in the liver and kidney tissues were determined by Biodiagnostic kits, according to the mentioned procedures.

**2.9. Assessment of the inflammatory markers by ELISA Technique**

ELISA kits were used to determine tumor necrosis factor-alpha (TNF-α), interleukin – 1 beta (IL-1β), interleukin – 2 (IL-2), interleukin – 6 (IL-6), and nuclear factor-kappa B (NF-κB) levels in the serum, liver and kidney tissues. Also, prostaglandin E2 (PGE2) levels were assessed in the liver and kidney tissues using rat ELISA KITS. Analysis was performed in line with the kits' manufacturers' protocols.

**2.10. Real-time-polymerase chain reaction**

Qiagen kit (USA) was used to extract the total RNA from the -80°C frozen tissues, which was then isolated and inversely transcripted into complementary DNA (cDNA), employing Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, USA). Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and an SYBR® Green PCR Master Mix (Applied Biosystems) were conducted in a 10 µl final volume, programming the heating cycles: 95°C (10 min), then 40 cycles of 95°C (15 s) and 65 °C (1 min). The sequences of PCR primer pairs with the corresponding bank gene accession number (Table 1). The results were estimated by the ABI Prism sequence detection system software and computed using v1.7 Sequence Detection Software, from PE Biosystems (Foster City, CA). Relative expression values of the studying genes were examined using the comparative threshold cycle method. All values were normalized to β-actin, applying the expression 2-ΔΔCt (Pfaffl, 2002).

**Table 1**: The sequences of PCR primer pairs with the corresponding bank gene accession number

| Gene | Primer | Sequences（5′→3′） | Gene bank accession number |
|---|---|---|---|
| iNOS | Forward | 5′-CCACAATAGTACAATACTACTTGG-3′ | NM_051345.1 |
| | Reverse | 5′-ACGAGGTGTTCAGCGTGCTCCACG-3′ | |
| NF-κB p65 | Forward | 5’-CCAAAGACCCACCTCACC-3’ | NC_005107.4 |
| | Reverse | 5′-TGGCTAATGGCTTGCTCC-3’-3′ | |
| COX-2 | Forward | 5′-CTGAGGGGTTACCACTTCCA-3′ | NC_000067.7 |
| | Reverse | 5′-TGAGCAAGTCCGTGTTCAAG-3′ | |
| TLR4 | Forward | 5′-CAGAATGAGGACTGGGTGAG-3′ | NC_051340.1 |
| | Reverse | 5′-GTTGGCAGCAATGGCTACAC-3′ | |
| MyD88 | Forward | 5′-TCCCACTCGCAGTTTGTT-3′ | NM_051343.1 |
| | Reverse | 5′-TGCCTCCCAGTTCCTTTG-3′ | |
| β-actin | Forward | 5′-CGGCAAGTTCAACGGCACAG-3′ | NM_051347.1 |
| | Reverse | 5′- CGCCAGTAGACTCCACGACAT-3′ | |

iNOS: inducible nitric oxide synthase, NF-κB p65: nuclear-factor - kappa B p65 subunit, COX-2: cyclooxygenase 2, TLR4: toll-like receptor 4, and MyD88: myeloid differentiation factor 88.

**2.11. Assessment of calcium level in the liver and kidney tissues**

The liver and kidney tissues of different studied groups were digested in a mixture of concentrated nitric acid ($HNO_3$) and hydrogen peroxide ($H_2O_2$) (5:1 v/v) until over-all digestion of the organic ingredients, using Milestone MLS-1200 Mega, High-Performance Microwave Digester Unit, Italy, at the RCFF. Calcium ($Ca^{2+}$) levels were assessed using ICP (OES), Perkin Elmer, Optima 2000 DV, at RCFF, Agricultural Research Center, Giza, Egypt.

**2.12. Statistical Analysis**

Data were inspected by the SPSS software program (Statistical Package for Social Science, version 21.0), which combined with Microsoft Excel. The results were demonstrated as the mean ± standard error (SE). One-way analysis of variance (ANOVA) the LSD (least significant difference) post hoc multiple comparisons were used to test the variation in the means of the variables among groups. The probability of $P < 0.001$ and $P < 0.05$ was thought to be significant.

## 3. Results

**3.1. Blood biochemical markers**

The LPS, IRR, and LPS/IRR-treated rats exhibited significant increases ($p<0.01$) in the activity of the hepatic enzymes: ALT, AST, ALP, LDH and GGT, and significant enhancement in the levels of TC, TG, urea, and creatinine ($p<0.01$), accompanied by significant declines ($p<0.01$) in HDL-Chol, ALB and total protein as compared to the controls. The LPS/IRR group displayed significant induction ($p<0.001$) in these biochemical parameters. However, LBE administration ameliorated these alterations

in the treated groups (LBE/LPS, LBE/IRR and LBE/LPS/IRR) comparing to the control ratios (Figure 1).

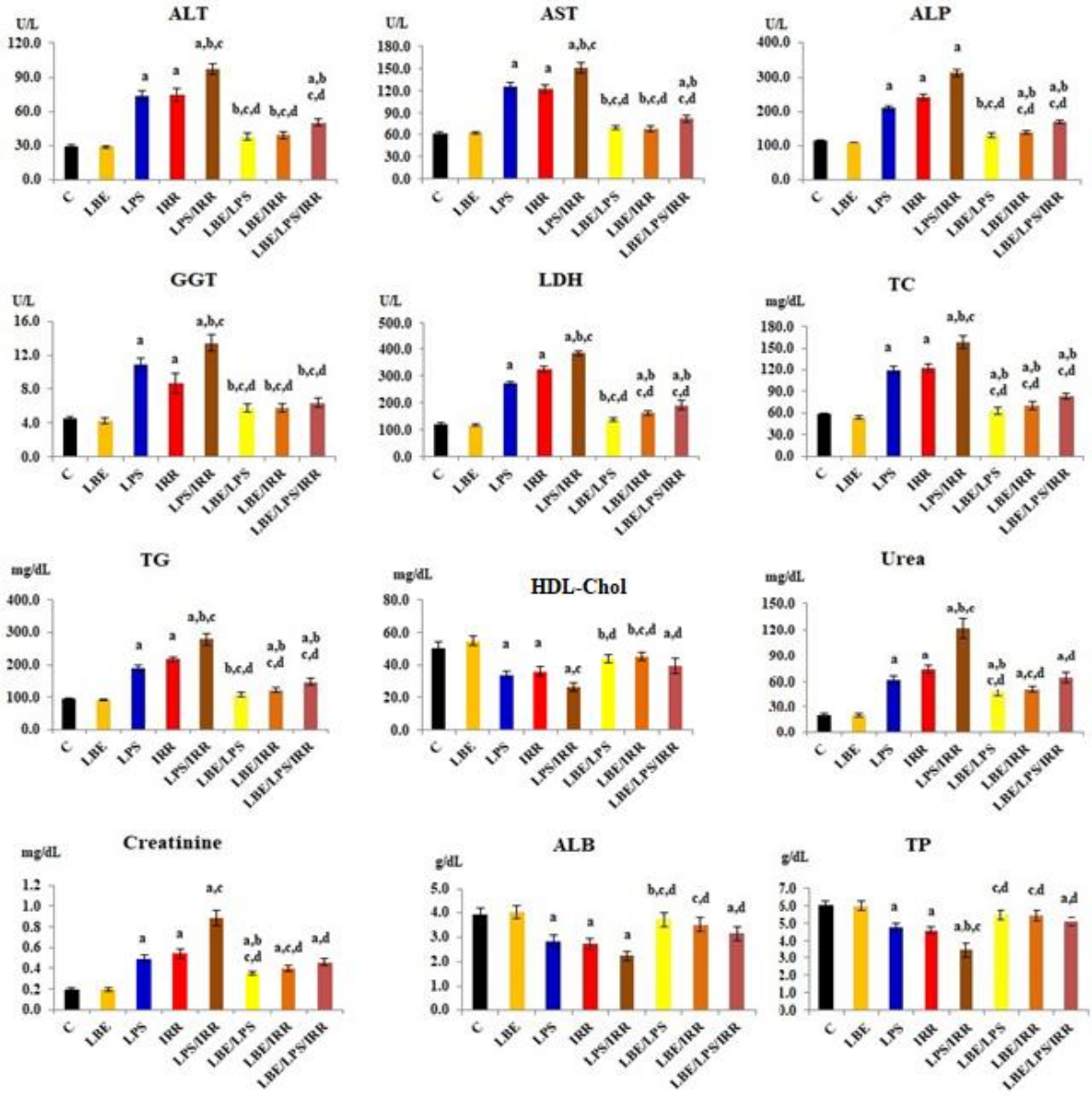


**Fig. 1:** Biochemical parameters in serum.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, ALT: alanine aminotransferase, AST: aspartate amino-transferase, GGT: Gamma-Glutamyl Transferase, LDH: lactate dehydrogenase, TC: total cholesterol, TG: triglycerides, HDL-Chol: high density lipoprotein-cholesterol, ALB: albumin, TP: total proteins. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

**3.2. Hematological indices**

The data established that RBCs, WBCs, and PLTs' counts, Hb concentration, and HCT percentage were reduced due to LPS injection, IRR exposure, and their combined toxic effects (LPS/IRR), as compared to their control values. On the other hand, the lymphocytes percentage was decreased (lymphocytopenia), while, the percentages of neutrophils, monocytes, and eosinophils were increased in the LPS, IRR, and LPS/IRR intoxicated groups. However, LBE treatment improved these hematologic indices in the treated groups (LBE/LPS, LBE/IRR and LBE/LPS/IRR) (Table 2).

**3.3. The oxidative stress and antioxidant function in the liver and kidney tissues**

The levels of MDA and NO were increased significantly, but the GSH contents were diminished, also, SOD, CAT, and GPX activities were inhibited in the liver and kidney tissues of the LPS, IRR and LPS/IRR treated rats regarding to control in the liver and kidney tissues. A considerable regulation of the MDA and NO levels, GSH contents and the activity of the antioxidant enzymes (SOD, CAT & GPX) in the liver and kidney tissues were observed in LBE treated groups; LBE/LPS, LBE/IRR and LBE/LPS/IRR, as compared to the LPS, IRR and LPS/IRR groups, respectively (Figure 2).

**3.4. The inflammatory markers**

The levels of TNF-α, IL-1β, IL-2, IL-6, and NF-κB were raised in serum; liver and kidney tissues of the LPS, IRR and LPS/IRR treated rats, as compared to the control group. LBE showed anti-inflammatory progress in contrast to the LPS, IRR and LPS/IRR treated groups via down-regulation of the investigated pro-inflammatory markers (Figure 3).

**3.5. Inducible nitric oxide synthase and nuclear-factor kappa B p65 subunit**

The relative gene expression ratios of iNOS and NF-κB p65 were upregulated in the liver and kidney tissues of LPS, IRR and LPS/IRR treated animals, as compared to the control values. LBE treated rats showed downregulation of iNOS and NF-κB p65gene expression ratios, as compared to LPS, IRR and LPS/IRR intoxicated groups (Figure 4).

**3.6. Cyclooxygenase 2 (COX-2)**

The relative gene expression ratio of COX-2 was upregulated in the liver and kidney tissues of LPS, IRR and LPS/IRR treated animals, as compared to the control values. LBE treated rats exhibited ameliorations of COX-2 gene expression ratios comparing to the corresponding control ratios in the LPS, IRR and LPS/IRR groups (Figure 5).

**3.7. Prostaglandin E2 (PGE2):**

The level of PGE2 was raised in the liver and kidney tissues of LPS, IRR and LPS/IRR treated animals, as compared to the control values. LBE treated rats exhibited ameliorations of PGE2 level, as compared to LPS, IRR and LPS/IRR groups (Figure 6).

**3.8. Toll-Like Receptor 4 and Myeloid Differentiation Factor 88**

The relative gene expression ratios of TLR4 and MyD88 were upregulated in the liver and kidney tissues of LPS, IRR and LPS/IRR treated animals, as compared to the control values. LBE-treated rats demonstrated downregulation of TLR4 and MyD88 gene expression ratios, as compared to LPS, IRR and LPS/IRR treated groups (Figure 7).

**3.9. Calcium level in the liver and kidney tissues**

The level of calcium was elevated in the liver and kidney tissues of LPS, IRR, and LPS/IRR treated animals, as compared to the control values. The level of calcium was reduced in the liver and kidney tissues of LPS, IRR and LPS/IRR groups treated with LBE, as compared to the corresponding intoxicated groups (Figure 8).

**Table 2:** Hematologic indices

| Group | RBC (× $10^6$/mm$^3$) | WBCs (× $10^6$/mm$^3$) | Platelets (× $10^3$/mm$^3$) | Hb (g/dL) | HCT (%) | Lympho-cytes (%) | Neutr-ophils (%) | Mono-cytes (%) | Eosino-phils (%) |
|---|---|---|---|---|---|---|---|---|---|
| **C** | 7.3 ± 0.51 | 5.5 ± 0.30 | 6.1 ± 0.43 | 13.7 ± 0.62 | 39.2± 1.25 | 37.8 ± 0.50 | 56.2 ± 1.25 | 4.8 ± 0.71 | 1.3 ± 0.29 |
| **LBE** | 7.9 ± 0.32 | 5.7 ± 0.35 | 6.5 ± 0.40 | 14.3 ± 0.78 | 41.8± 1.7 | 38.6 ± 2.35 | 55.3 ± 2.25 | 4.6 ± 0.68 | 1.5 ± 0.3 |
| **LPS** | 4.8 ± 0.36 [a] | 3.2 ± 0.39 [a] | 4.1 ± 0.33 [a] | 7.8 ± 0.48 [a] | 24.8± 0.71 [a] | 14.6 ± 1.53 [a] | 70.1 ± 1.51 [a] | 6.7 ± 0.87 | 8.6 ± 0.71 [a] |
| **IRR** | 5.1 ± 0.31 [a] | 2.4 ± 0.23 [a] | 3.1 ± 0.24 [a] | 8.7 ± 0.45 [a] | 26.5± 0.89 [a] | 16.4 ± 2.36 [a] | 68.4 ± 3.00 [a] | 7.9 ± 0.68 [a] | 7.3 ± 1.239 [a] |
| **LPS/IRR** | 3.8 ± 0.19 [a,c] | 1.9 ± 0.21 [a,b] | 2.4 ± 0.31 [a,b] | 5.9 ± 0.53 [a,b,c] | 15.7 + 0.82 [a,b,c] | 5.9 ± 0.87 [a,b,c] | 80.3 ± 0.91 [a,b,c] | 7.2 ± 1.29 [a] | 6.5 + 0.97 [a,b,c,d] |
| **LBE/LPS** | 6.1 ± 0.43 [a,b,d] | 4.8 ± 0.41 [b,c,d] | 5.1 ± 0.25 [b,c,d] | 11.1 ± 0.59 [a,b,c,d] | 33.1 ± 2.33 [a,b,c,d] | 27.2 ± 2.65 [a,b,c,d] | 63.1 ± 3.23 [a,b,d] | 5.9 ± 0.40 | 3.8 ± 0.60 [a,b,c,d] |
| **LBE/IRR** | 6.1 ± 0.35 [a,b,c,d] | 3.4 ± 0.37 [a,c,d] | 4.8 ± 0.42 [a,c,d] | 11.8 ± 0.56 [a,b,c,d] | 33.4 ± 2.07 [a,b,c,d] | 27.7 ± 2.23 [a,b,c,d] | 62.6 ± 2.93 [b,d] | 5.9 ± 0.51 | 4.1 ± 0.39 [a,b,c,d] |
| **LBE/LPS/IR** | 4.9 ± 0.33 [a,c] | 3.1 ± 0.28 [a,d] | 3.9 ± 0.35 [a,d] | 9.5 ± 0.64 [a,d] | 26.2 ± 1.75 [a,d] | 22.2 ± 2.45 [a,b,d] | 65.9 ± 2.51 [a,d] | 6.3 ± 0.78 | 5.6 ± 0.64 [a,B] |

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation. RBCs: Red blood cells, WBCs: white blood cells, PLT: platelets, Hb: hemoglobin, and Hct: hematocrit. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 6 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

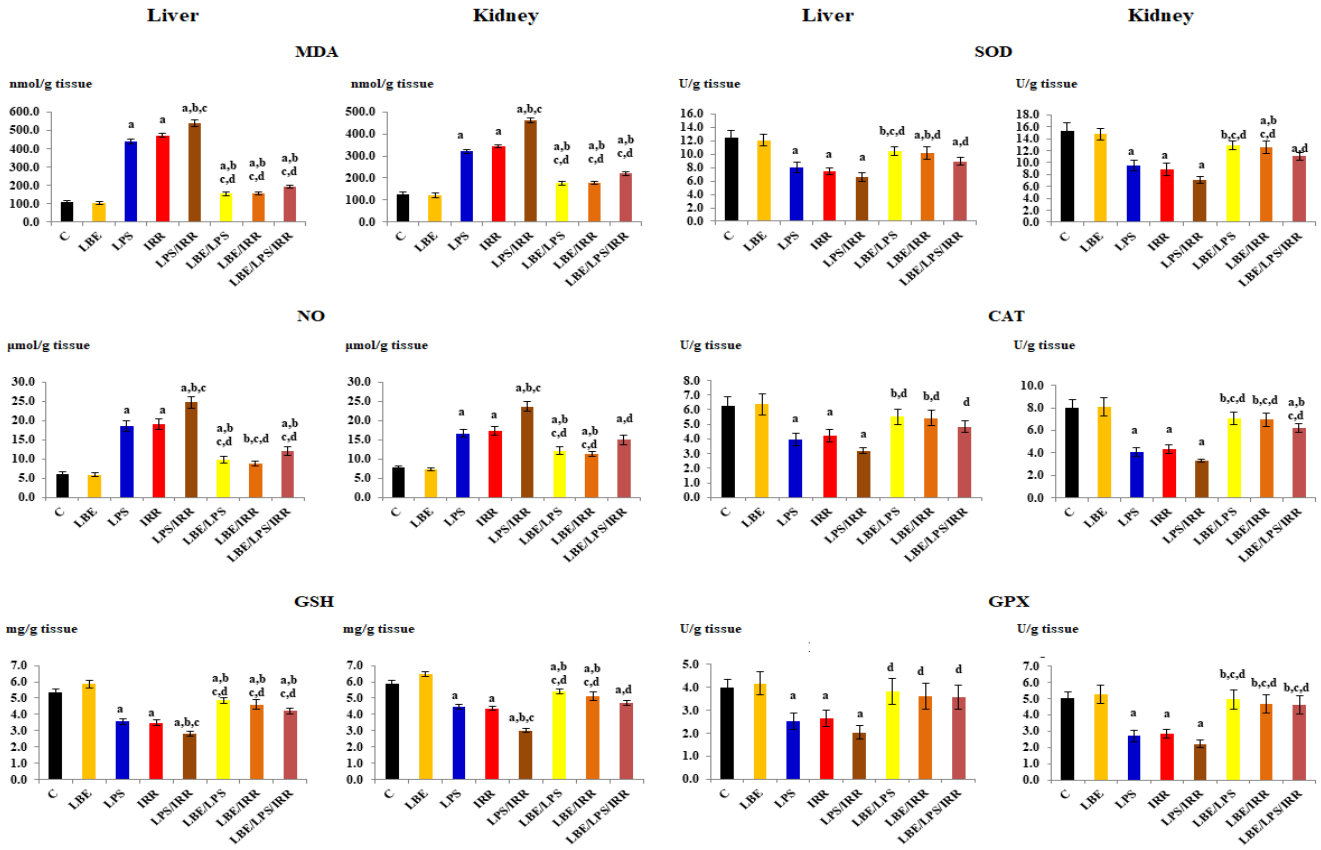


**Fig. 2:** The oxidative stress and antioxidant function in the liver and kidney tissues

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, MDA: malondialdehyde, NO: nitric oxide, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, and GPX: glutathione peroxidase. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

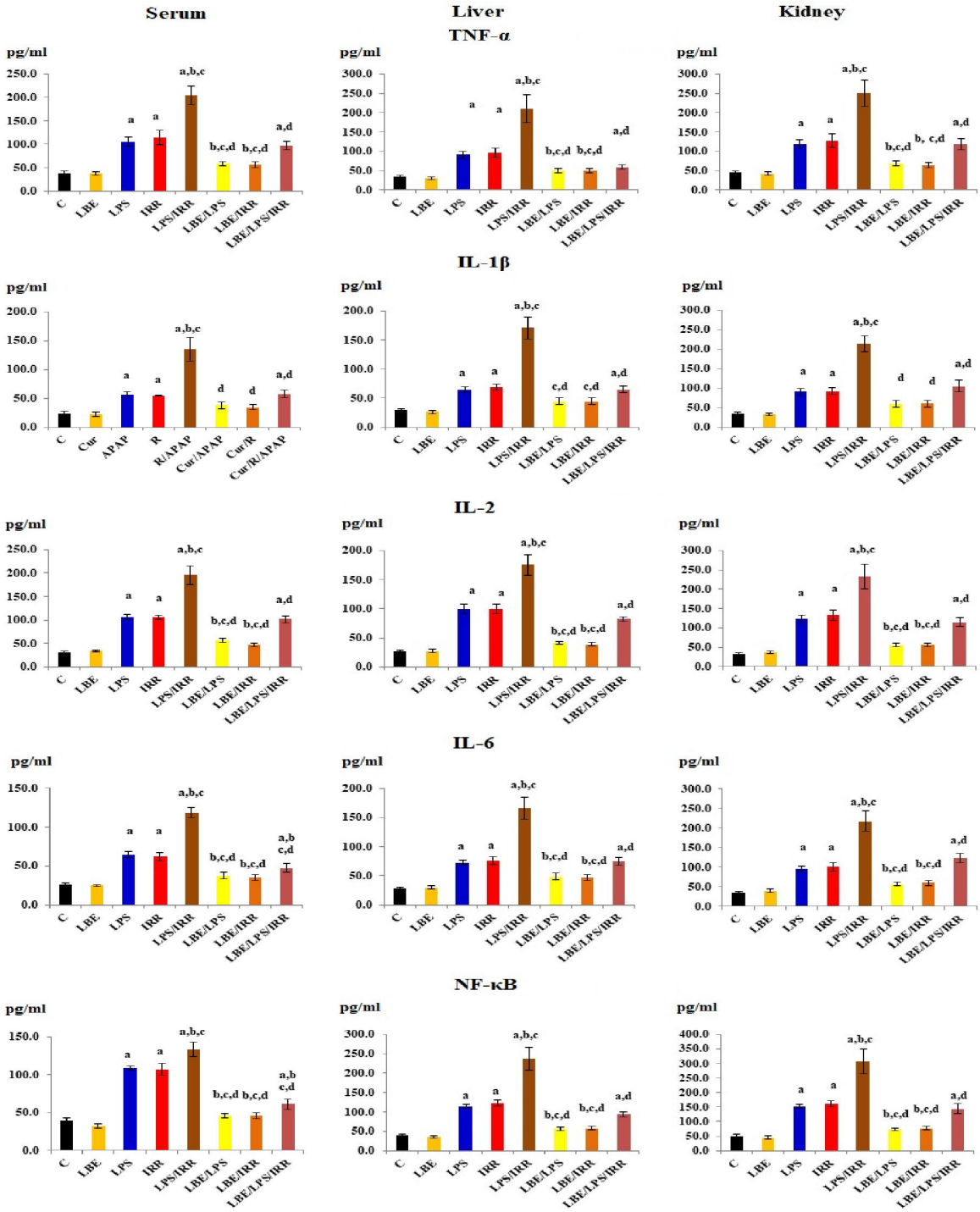


**Fig. 3:** The inflammatory markers levels in the serum, liver and kidney tissues.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, TNF-α: Tumor necrosis Factor alpha, IL-1β: Interleukin 1 beta, IL-2: Interleukin 2, IL-6L Interleukin, NF-κB: Nuclear Factor kappa B. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

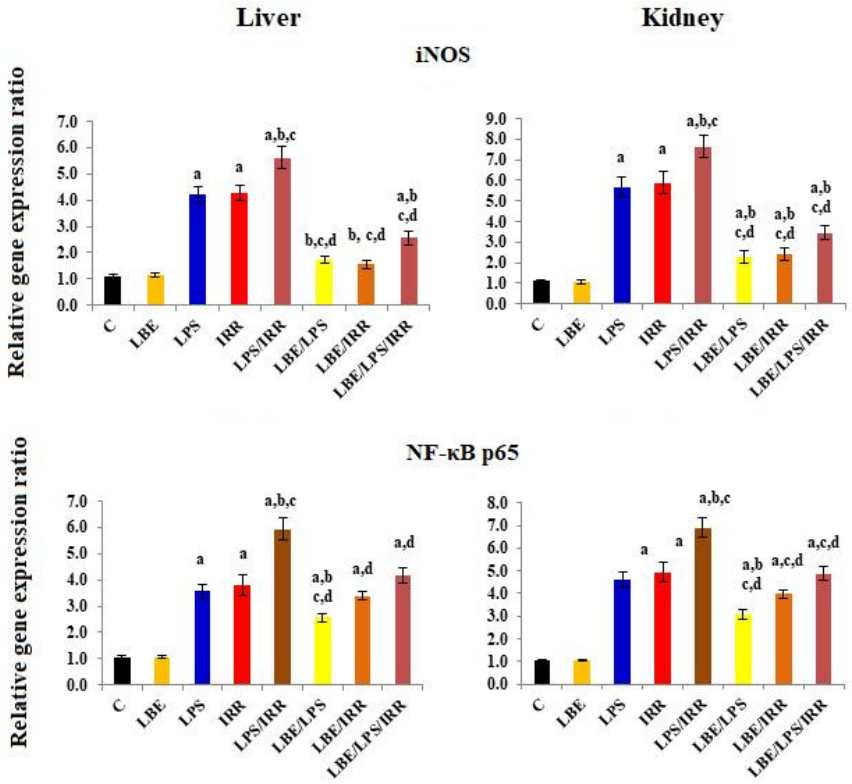


**Fig. 4:** Relative gene expression ratios of inducible nitric oxide synthase and nuclear-factor kappa B p65 subunit in the liver and kidney tissues.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, iNOS: inducible nitric oxide synthase, NF-kB p65: nuclear-factor kappa B p65 subunit. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

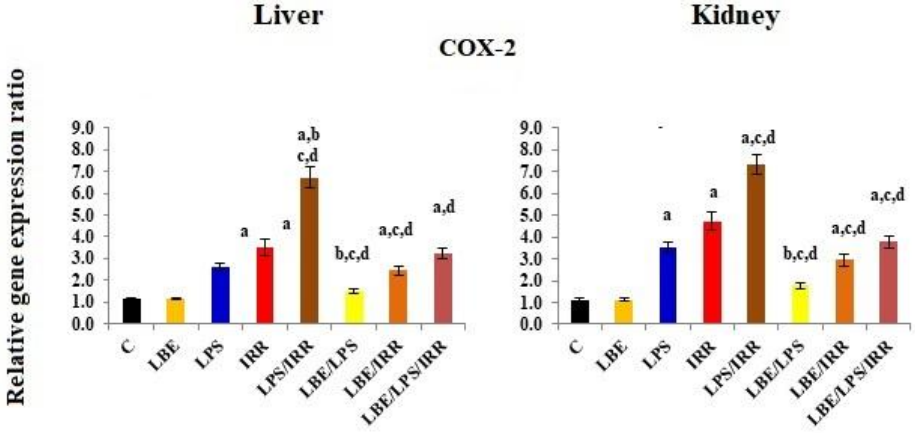


**Fig. 5:** Relative gene expression ratios of cyclooxygenase-2 in the liver and kidney tissues.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, COX-2: cyclooxygenase-2. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

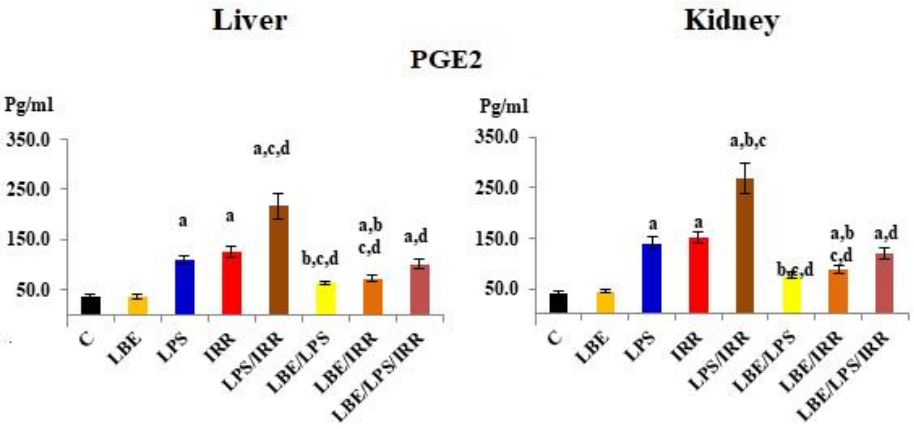


**Fig. 6:** The level of prostaglandin E2 in the liver and kidney tissues.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, PGE2: prostaglandin E2. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

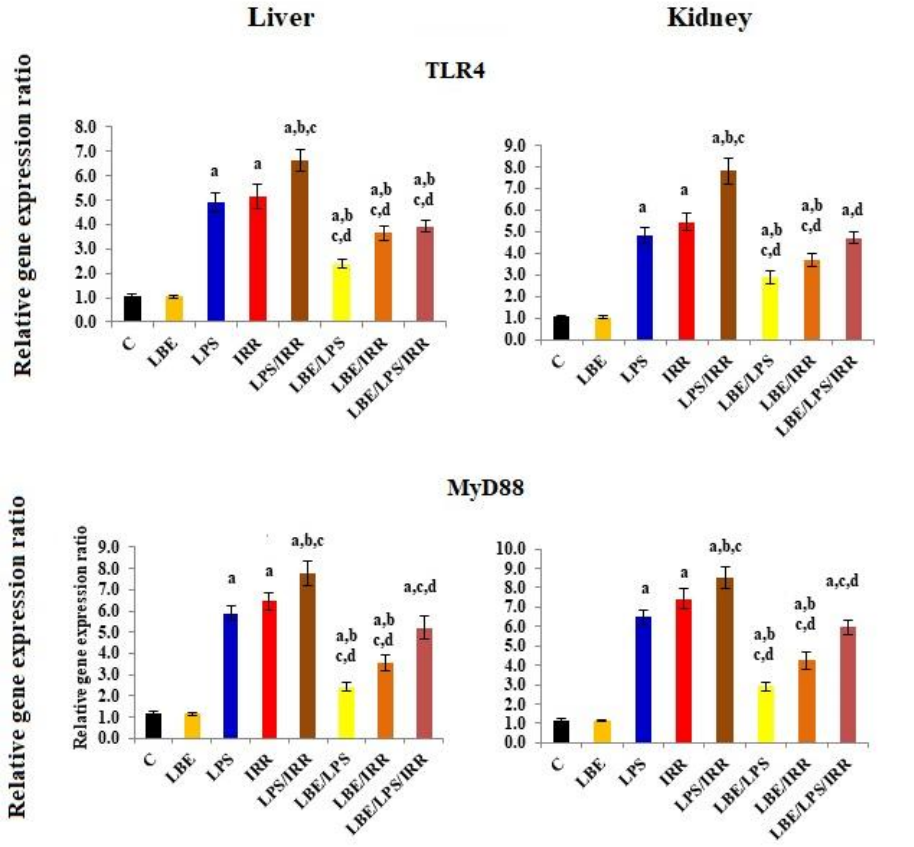


**Fig. 7:** Relative gene expression ratios of toll-like receptor 4 and myeloid differentiation factor 88 in the liver and kidney tissues.

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation, TLR4: toll-like receptor 4, MyD88: myeloid differentiation factor 88. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

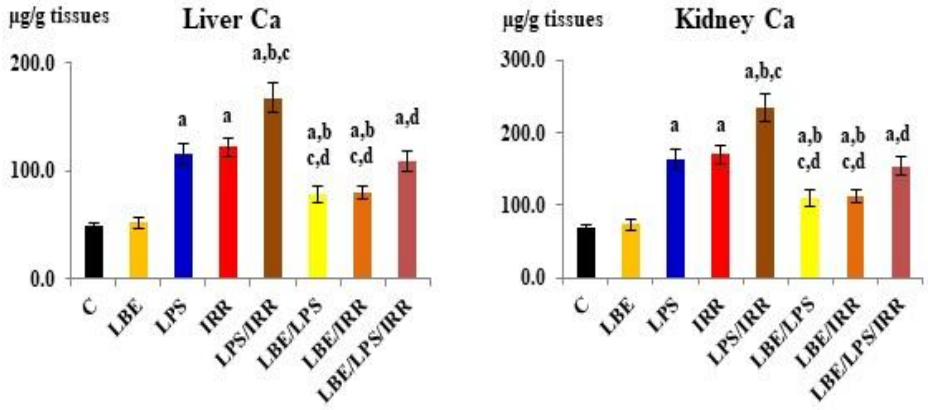


**Fig. 8:** The levels of calcium in the liver and kidney tissues

C: control, LBE: *Lycium barbarum* extract, LPS: lipopolysaccharides, IRR: gamma-irradiation. The results are demonstrated as mean ± standard error (SE), n = 12 rats/group. While the conducting experiment, one rat was died from LPS, another one from IRR, while two rats were passed away from LPS/IRR group. Accordingly, the results of 10 animals from each group were used in the statistical analysis. a: significant to the control, b: significant to LPS, c: significant to IRR, and d: significant to LPS/IRR at $p < 0.01$ and $p < 0.001$.

## 4. Discussion

Results obtained showed a marked increase in the hepatic enzymes (ALT, AST, ALP and GGT) due to the extensive hepatic damage induced by LPS and /or IRR groups. Similar results were demonstrated previously in LPS intoxicated (Khan *et al*., 2021) and in gamma-irradiated rats (Eassawy *et al*., 2021, and Hassan *et al*., 2021). The increase in the hepatic enzymes activity is resulted in the loss of functional integrity of the hepatic membrane due to oxidative damage, causing the leakage of cellular enzymes (Mohamadin *et al*., 2011). Besides, oxidative stress convinces biliary obstacle, thus enhancing the synthesis of ALP, which is existed in the canalicular and luminal area of the bile duct epithelium, thus released into the blood stream (Limdi *et al*., 2003). Moreover, the oxidative stress that provokes damage of the hepatocytes and its membrane, as well as, the generated hypoxia, enhanced the levels of LDH in the blood stream (Kotoh *et al*., 2008).

Meanwhile, in accordance to the previous work (Wahby *et al*., 2015, Darwish *et al*., 2007), the serum levels of TC, TG, urea and creatinine were significantly elevated while HDL-cholesterol, TP and ALB levels were decreased in the LPS, and IRR treated rats. The observed hyperlipidemia in irradiated-rats is due to the motivation of hepatic enzymes that induced the fatty acids biosynthesis and the of adipose tissues release fats into the blood stream (Darwish *et al*., 2007). Hyperlipidemia is considered a protection mechanism against LPS toxicity. The serum lipoproteins are combined with LPS to downgrading the toxicity (Van Oosten *et al*. 2001). The body protective mechanism to oppose LPS toxicity is also drives to decay in HDL-cholesterol quantity (Khan *et al*., 2021). On the other hand, the elevated levels of urea and creatinine after LPS administration or IRR exposure pointed out the renal impairment, as indicated previously (Salem and Ismail, 2021, Prince *et al*., 2017). Oxidative stress instigates oxidative de-amination of amino acids, destruction of proteins, and membranes, modifications of the membranes' permeability, and tubular epithelium and other tubulointerstitial components impairment, causing kidney deterioration and electrolyte-imbalance (Salem and Ismail, 2021). However, the change in the serum proteins level is a direct response to LPS administration (Yilmaz *et al*., 2016), and exposure to gamma-irradiation (Eassawy *et al*., 2021, El-Khafif *et al*., 2003). Because liver is the manufacture of the blood proteins, liver injury and the obtained oxidative damage demonstrates alterations in the serum proteins levels, resulted in de-natured proteins, so the levels of TP is declined in the serum (Eassawy *et al*., 2021, El-Khafif *et al*., 2003). The investigated biochemical parameters were augmented in the serum of the LPS/IRR treated rats. However, LBE administration revealed improvement of the levels of these biochemical parameters in the LPS. IRR and LPS/IRR intoxicated groups. LBE administration demonstrated hepatoprotection against acetaminophen toxicity (Gündüz *et al*., 2015, Lee *et al*., 2021), and renoprotection on lead

toxicity **(**Xie *et al*., 2021), by recovering the hepatic and renal functions, and lipid metabolism, mainly due to the antioxidant defensive impacts.

The data confirmed that RBCs, WBCs, and PLTs' counts, Hb concentration, and HCT percentage were downgraded owing to LPS toxicity, IRR exposure, and augmented due to their combined toxic effects (LPS/IRR). In addition, lymphopenia and neutrophilia are observed in the blood of LPS, IRR and LPS/IRR intoxicated rats, whereas the percentages of lymphocytes and that of neutrophils were reduced, as well as monocytes and eosinophils percentages were increased in the blood. These results are inconsistent with the pervious researches (Abojassim *et al*., 2015, Brauckmann *et al*., 2016, and Zaher *et al*., 2016). The blood cells displayed a critical role during inflammation. LPS can triggered hemolysis, leading to mortality. LPS combines to the Toll-like receptor (TLR)-4 and stimulates the innate immune, thus triggering inflammation and motivating coagulation process. Blood hemolysis is mediated by the toxicity of hemoglobin and its degradation products. Moreover, LPS combines to the red cell membrane, drawing out red cell membrane impairment and hemolysis (Brauckmann *et al*., 2016). LPS infusion and encourage platelets' aggregations and quick reductions in the circulating platelet count (Zhao *et al*., 2002). In contrast, the hematopoietic elements are radio-sensitive, gamma-radiation has the capability to damages hematopoietic system through destructions of the splenic hematopoietic stem cells (Abojassim *et al*., 2015, and Zaher *et al*., 2016). Neutrophils are the leukocytes in the blood that involved in the defense during tissue injury. However, in such cases, forced activation of neutrophils induced chronic inflammation and dysfunction of different organs (Xie *et al*., 2021 & Ozánska *et al*., 2020). Monocytes are related to the mononuclear phagocyte system, representing most effective constituents of the innate immunity. Monocytes are leukocytes that derived from bone marrow and released to the blood. Monocytes have the potency to identify the hazardous signs during tissue damage. Thus, monocytes can display different functions via secretion of chemokines that play an important role in phagocytosis and cells proliferation, also can be inspired to macrophages and dendritic cells (Chiu and Bharat, 2016). On the other hand, inflammation suppression resulted in regulation of leukocytes balance and inspires of repair of damaged tissues (Ozánska *et al*., 2020 & Azambuja *et al*., 2022). However, LBE administration exhibited obvious improvement of these hematologic indices in the treated groups (LBE/LPS, LBE/IRR and LBE/LPS/IRR).

The data of the current investigation demonstrated raised levels of oxidative stress in the liver and kidney tissues of LPS and IRR groups, verified by elevated levels of MDA and NO coexistent with dimensioned GSH levels and suppressed antioxidant enzymes' activity. The suppressions of the body antioxidant system bring about oxidative stress is concomitant by release of ROS (Baynes and Thorpe, 1999). The observed oxidative stress was improved in the LPS/IRR treated animals. SOD, CAT and GPX are extremely the central antioxidant system in different organisms. They associated to protect different organs against ROS liberation, reduction of peroxides, and preserving the redox balance. MDA, the fundamental lipid peroxides, overexpression denotes oxidative stress events (Xu *et al*., 2021). There are strong evidences that LPS prompted liver and kidney injury. LPS triggered oxidative stress via induction of significant levels of hepatic MDA, and suppressed the antioxidant system, as reported previously (Yang *et al*., 2017, Xu *et al*., 2021). Likewise, LPS administration engendered renal ROS (Shi *et al*., 2019), due to glomeruli and tubules damage, causing kidney and renal impairments (Mukhopadhyay *et al*., 2018). On the same direction, exposure to IRR triggered oxidative stress via provocation of the levels of hepatic and renal MDA, and suppressed the liver and kidney antioxidant system (Hassan *et al*., 2021, Azab *et al*., 2017, Salem and Ismail, 2021). IRR exposure instigates water radiolysis in different tissues, concomitant with release of hydroxyl radicals ($^{\bullet}OH$) that attach the biological membranes' polyunsaturated fatty acids fraction, thus damaging the cell membranes (Hassan *et al*., 2021). On the other hand, there is a great suggestion that the NO pathway is critically implicated in the infections and organ injury, inducing nitrosative stress and inflammation. The data showed significant increase of NO level with upregulation of iNOS gene expression in the liver and kidney tissues of LPS and IRR intoxicated rats. LPS and IRR trigger the activation of iNOS, which causing the production of high level of NO (Bekpinar *et al*., 2014, Ismail *et al*., 2016). NO demonstrates antimicrobial and cytotoxic actions during inflammation, but concomitant with adverse effects and damage of different organs, including liver and kidney, due to peroxynitrite ($ONOO^-$) formation (Ismail *et al*., 2016, Mori and Gotoh, 2000). However, LBE administration demonstrated regulation of the antioxidant system, via activation of the antioxidant enzymes (SOD, CAT, nd GPX), suppressed the release of MDA, NO and down-regulated iNOS gene expressions in the liver and kidney tissues of LPS,

IRR and LPS/IRR intoxicated groups, due to its highest antioxidant defensive force and free radicals scavenging activity in the hepatic and renal tissues (Gündüz *et al*., 2015, Lee *et al*., 2021, Xie *et al*., 2021).

The data demonstrated that the levels of TNF-α, IL-1β, IL-2, IL-6, and NF-κB were elevated; as well as the relative gene expression ratios of NF-κB p65 were upregulatedin the serum, liver and kidney tissues of the LPS, IRR and their values were augmented in the LPS/IRR group. Exposure to LPS or IRR provokes the progression of inflammatory conditions and release of the inflammatory markers in the serum and different organs (Khan *et al*., 2021, Ismail *et al*., 2016, Salem and Ismail, 2021, Eassawy *et al*., 2021, Zhang *et al*., 2021). Acute or chronic inflammation causes parenchymal and renal cells damage, leading to metabolic syndromes and organs malfunction (Khan *et al*., 2021). At relaxation conditions, NF-κB p65 and the inhibitory protein IκB are connected with each other, which derive NF-κB in a passive state in the cytoplasm. Under oxidative stress, IκB, which is promptly phosphorylated and detached from NF-kBp65 translocates into the nucleus, accordingly the NF-κB and its downstream pathways are activated (Wang *et al*., 2017, Nežic´ *et al*., 2019).

LPS and IRR are associated to improve the oxidative stress and inflammatory yield in LPS/IRR intoxicated animals. Moreover, the relative gene expression ratios of COX-2 and PGE2 were upregulated in the liver and kidney tissues of LPS, IRR and LPS/IRR treated animals. In the course of an inflammatory response to LPS or IRR provocation of PGE2 is attributed to COX-2 aggravation in tissues. At beginning point in the inflammatory reaction, NF-κB translocate into the nucleus, connects to the promoter region of COX-2, causing the instruction of COX-2 (Tsukayama *et al*., 2021, Azab *et al*., 2017). The data also indicated that the relative gene expression ratios of TLR4 and MyD88 were upregulated in the liver and kidney tissues of LPS, and IRR treated animals, which were improved in the LPS/IRR co-treated animals. Activation of TLR4 is a protective mechanism to safeguard the cells against the inflammatory response of IRR (Mehdipour *et al*., 2021). LPS encourages the activation of TLR4 signaling pathway through activation of MyD88. (Khan *et al*., 2021). The outcomes of activation of TLR subsequent stimulation of NF-κB and release of TNF-α, IL-6, IL-1β, conducting liver and kidney damage (Khan *et al*., 2021, & Zhang *et al*., 2021). The administration of anti-inflammatory drugs to suppress the release of different inflammatory markers is associated with antagonistic output that destroys different organs (Khan *et al*., 2021). Consequently, investigation of safer treatments is an essential target to protect the body organs against inflammation and oxidative stress. In the current investigation, the administration of LBE showed anti-inflammatory inspirations in contrast to the LPS, IRR and LPS/IRR treated groups via down-regulation of TLR4/MyD88 pathway, leading to suppression of NF-κB, and consequently squashing of COX-2/PGE2 and the investigated pro-inflammatory cytokines.

The data showed elevated levels of calcium in the liver and kidney tissues of LPS and IRR groups, which was expanded in the LPS/IRR co-treated rats. Calcium level was raised in macrophage of mice treated with LPS (Lo and Lo, 2013). Many clinical investigations have demonstrated an increase in free intracellular calcium in patients with sepsis (Bhattacharyya and Sayeed, 1997, Hotchkiss and Karl, 1996). Moreover, calcium levels were increased in the liver and kidney of rats exposed to gamma-irradiation (Eassawy *et al*., 2021, & Salem and Ismail, 2021). Calcium mediated several signaling pathways, including inflammatory mediators' gene expression, thus it has an imperative role in cellular functions, and participated in the pathophysiology role of LPS toxicity (Ermak and Davies, 2001). The cellular surface receptors encourage the release of calcium under LPS stress. Elevated levels of intracellular calcium inspire LPS to produce IL-6 and TNF-α (Lo *et al*., 1996, Lo and Lo, 2013). So, regulation of intracellular calcium level can resist the release of the pro-inflammatory cytokines (Lo and Lo, 2013). The administration of LBE demonstrated a significant regulation of calcium levels in the LPS, IRR and LPS/IRR groups, which could be a juncture to control the release of the inflammatory markers.

## 5. Conclusion

The results demonstrated that LBE exhibited protective function by preserving the liver and kidney tissues from the oxidative stress and the inflammation induced by LPS and/or IRR, mediated by regulation of calcium level and COX-2/PGE2/TLR4/MyD88/NF-κB pathway.

**Credit authorship contribution statement**

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Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, and review.

**Amel F.M. Ismail:**
Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review and editing.

**Declaration of competing interest**

The authors declare that they have no conflict of interest.

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