



Effect of *Lactobacillus casei* on Oxidative Stress, Biochemistry, and Renal Gene Expression in HgCl₂-Exposed Rats

Milad Amrollahi¹, Saba Taheri^{1,2}, Behnaz Esfandiari^{1,2*}

1. Department of Biology, ISI.C, Islamic Azad University, Islamshahr, Iran.

2. Institute of biosocial and quantum science and technologies, ISI. C., Islamic Azad University, Islamshahr, Iran.

Corresponding Author: Behnaz Esfandiari, PhD in Biology, Institute of biosocial and quantum science and technologies, ISI. C., Islamic Azad University, Islamshahr, Iran. P.C: 33147-6763. Email: Behnaz.esfandiari@iiiau.ac.ir

Received ; 2025/05/10 **Accepted** 2025/07/13

Abstarct

Introduction: Mercuric chloride (HgCl₂) disrupts kidney function by inducing oxidative stress and inflammation. Probiotics, such as *Lactobacillus casei*, may mitigate these effects. This study investigates the impact of *L. casei* on renal function, oxidative stress markers, and inflammatory/apoptotic gene expression in HgCl₂-exposed Wistar rats. **Methodology:** Twenty-eight rats were divided into four groups: control, HgCl₂-treated, *L. casei*-treated, and combined treatment. Serum levels of urea, creatinine, and antioxidant enzymes (SOD, CAT, and MDA) were assessed. Expression of TNF-α, IL-6, Bcl-2, and Bax genes was analyzed using real-time PCR. Histopathological changes were also examined. Statistical analysis was performed using one-way ANOVA (P > 0.05).

Results: Exposure to HgCl₂ significantly increased urea, creatinine, and MDA levels by >100% (P < 0.0001) and reduced SOD and CAT activities by 26% (P > 0.05 vs. control) and 92% (P < 0.0001), respectively. Co-treatment with *L. casei* partially restored SOD (13.5% improvement, P > 0.05 vs. toxin) and CAT (42% improvement, P < 0.05 vs. toxin). Probiotics also significantly downregulated IL-6, TNF-α, and BAX (P < 0.05) and upregulated BCL2 (P < 0.05). Additionally, glomerular size was restored by 59% (P < 0.0001 vs. toxin).

Conclusion: *L. casei* exhibited protective effects against HgCl₂-induced renal injury by reducing oxidative stress, inflammation, and apoptosis, suggesting its potential as a therapeutic agent in nephrotoxicity management.

Keywords: Lactobacillus casei, Mercuric chloride, Antioxidant Enzymes, Apoptotic Genes, Probiotic,

1. Introduction

HgCl₂ is a highly toxic compound that poses significant health risks when introduced into the body (1). As an inorganic form of Hg, it can enter the system through various routes, including ingestion, inhalation, or dermal exposure (2). Once absorbed, HgCl₂ disseminates throughout the body, exerting its harmful effects on multiple organs, particularly the kidneys (3). The kidneys are crucial for filtering waste and maintaining fluid and electrolyte balance; however, they are also susceptible to damage from toxic substances (4). HgCl₂ induces nephrotoxicity primarily through oxidative stress, inflammation, and cellular apoptosis (5).

The compound disrupts cellular functions and causes structural damage to renal tissues, leading to a decline in kidney function (6). The pathophysiological impact of HgCl₂ on kidney tissue manifests as glomerular injury, tubular degeneration, and interstitial fibrosis (7). These changes can compromise the kidneys' ability to filter blood effectively, resulting in various complications, including proteinuria, electrolyte imbalances, and acute kidney injury (8). Understanding the toxicity of HgCl₂ is essential for developing effective treatments and preventive measures against Hg poisoning. HgCl₂ exposure significantly alters biochemical markers and gene expressions related to kidney function and oxidative stress (9). One of the primary indicators of kidney impairment is the elevated serum levels of creatinine and urea, which are commonly observed following mercuric HgCl₂ (10).

These substances accumulate in the bloodstream due to the kidneys' impaired ability to filter waste products effectively. In addition to renal function markers, HgCl₂ exposure influences oxidative stress parameters. Notably, levels of SOD and catalase CAT—two critical antioxidants that protect cells from oxidative damage—are often reduced in the presence of HgCl₂ (11). This decline suggests a compromised antioxidant defense mechanism, leading to increased oxidative stress (12). Conversely, MDA levels typically rise, indicating heightened lipid peroxidation and cellular damage (13). At the molecular level, HgCl₂ affects the expression of several key genes involved in apoptosis and inflammation. The pro-inflammatory cytokines *TNF-α* and *IL-6* are frequently upregulated in response to mercury exposure, reflecting an inflammatory response within the renal tissue (14). Simultaneously, the expression of the anti-apoptotic gene *Bcl-2* may decrease, while the pro-apoptotic gene *Bax* tends to increase, promoting cellular apoptosis (15). These alterations in gene expression contribute to the overall nephrotoxic effects of HgCl₂, leading to further renal injury and dysfunction (16).

Probiotics are live microorganisms that, when consumed in sufficient quantities, provide health benefits (17). They have gained interest for their potential role in supporting kidney health. By fostering a balanced gut microbiome, probiotics can significantly influence systemic inflammation and oxidative stress, both of which are crucial for maintaining renal function (18).

Research indicates that probiotics may help protect against kidney damage, particularly from toxins like HgCl₂. These beneficial bacteria may enhance kidney function by increasing antioxidant activity and reducing inflammatory responses (19). A healthier gut microbiome can lead to a decrease in harmful metabolites, which helps improve serum creatinine and urea levels, suggesting better renal performance (20). On a biochemical level, probiotics are thought to enhance the activity of key antioxidant enzymes, such as SOD and catalase CAT (21). This boost helps combat oxidative stress, resulting in lower levels of MDA, a marker of lipid peroxidation and cellular damage (22).

Additionally, probiotics can influence the expression of inflammatory cytokines and genes related to apoptosis. They may reduce the levels of pro-inflammatory factors like *TNF-α* and *IL-6*, thus lessening inflammation in the kidneys (23). Furthermore, probiotics may help shift the expression balance of apoptosis-related genes, potentially increasing *Bcl-2* (an anti-apoptotic factor) while decreasing *Bax* (a pro-apoptotic factor) (24). This change can protect renal cells from apoptosis and support kidney function. In this context, this study aimed to investigate the effect of *L. Casei* probiotics on serum levels of creatinine, urea, SOD, CAT, and MDA, as well as the expression of the apoptotic gene *Bax*, the anti-apoptotic gene *Bcl-2*, and finally, the genes encoding the cytokines *TNF-α* and *IL-6* in the kidney tissue of rats exposed to HgCl₂.

2. Materials and Methods

2.1. Study Population, Conditions, and Groups of Rats

The probiotic powder of *L. casei* (IBC-M10784, Takgene Zist Co., Iran) was suspended in distilled water to obtain a concentration of 10¹⁰ CFU/mL.

This suspension was administered daily to the rats along with their feed. For the toxicological challenge, HgCl₂ (code 23920, Alpha Aesar Co., Germany; alternatively sourced from Sigma-Aldrich, Germany) was dissolved in sterile distilled water at a concentration of 1 mg/mL. Rats received HgCl₂ via intraperitoneal (IP) injection at a dose of 3 mg/kg body weight, administered in a volume of 1 mL per 100 g body weight. This dosage regimen is well-established in nephrotoxicity studies (25).

A total of 28 male adult Wistar rats, averaging 200–250 g in weight, were purchased from the Pasteur Institute and were maintained under 12 h of light and 12 h of darkness at a temperature of 22 ± 3°C and 70% humidity. They were then randomly divided into four groups of seven. The first group served as the control, receiving only water and food daily. The second group consisted of rats receiving HgCl₂ (3 mg/kg). The third group received *L. casei* (10⁹ cfu/ml), and the fourth group received both HgCl₂ (3 mg/kg) and *L. casei* (10⁹ cfu/ml) simultaneously. HgCl₂ was administered three times with a one-day interval via intraperitoneal injection, and probiotics were gavaged for 30 days. After the treatment period, the rats were anesthetized using ketamine (87 mg/kg) and xylazine (13 mg/kg) via intraperitoneal injection (26), and direct blood sampling from the heart was performed. Intestinal and kidney tissues were collected for histological studies, gene expression analysis, and antioxidant factor evaluation. Blood samples were centrifuged at 4000 rpm (Behdad Co, Iran) for 10 min at room temperature to separate the serum, which was stored at -20°C until testing.

2.2. Assessment of Biochemical Parameter Levels

The level of serum creatinine was measured using the Jaffe method, which involved the formation of a yellow-orange color complex that was read at a wavelength of 510 nm. Furthermore, urea was measured by assessing ammonium levels through the Berthelot reaction (27) and the enzymatic test of glutamate dehydrogenase using spectrophotometry by ELISA reader at a wavelength of 340 nm (Stat Fax 2100, Awareness Technology, USA). For measuring sodium and potassium levels, photometric methods and Ion Selective Electrode (ISE) (28) techniques were employed, not photometric methods. Measurements were performed using the EasyLyte Electrolyte Analyzer (Medica, USA), calibrated daily with standard solutions. Calcium levels were assessed using the "ortho-cresol phthalein" method (29), a widely used technique, where OD was read in a spectrophotometer after 5 min. Finally, uric acid levels were measured using the uricase method (30), which is known for its high sensitivity.

2.3. Assessment of Antioxidant Activity in Samples

Antioxidant kits, including those for measuring SOD, CAT, and MDA, were purchased (ZellBio GmbH Co, Germany). Kidney tissues were first extracted and stored at -70°C until evaluations were conducted. The homogenized tissue was used to determine SOD activity based on the methodology of Paoletti, using spectrophotometry (31). Tissue samples were lysed using the buffer provided in the kit (Shokouh Pars Azmoon Co, Iran). For every 100 mg of tissue, 500 µl of lysis buffer was added and homogenized. The samples were then centrifuged at 10,000 rpm for 5 min at 4°C, and the supernatant was collected for analysis.

The supernatant was mixed with the solutions and reagents in the kit and added to a 96-well plate. After 5 min of keeping at an incubator (Aryateb Co, Iran), the optical density (OD) of the samples was read at a wavelength of 405 nm using a microplate reader (Thermo Fisher Scientific Co, USA), and calculated using the following formula.

SOD activity (U/ml or mg protein) = (OD Test/OD Control) × 200. The MDA level was assessed as a marker of lipid peroxidation using chromogenic reagents (32). 50 mg of tissue was homogenized, and then 500 µl of RIPA (Radio-Immunoprecipitation Assay) buffer was added for lysis. After 1 h of refrigeration, the samples were centrifuged at 10,000 rpm for 15 min, and the supernatant was transferred to new microtubes. According to the kit protocol, the supernatant was mixed with the kit solutions and then centrifuged again. After transferring to the wells of the 96-well plate, the OD was measured at 535 nm, and the concentration was calculated based on the obtained standard curve. The method developed by Beers (33) was used for measuring CAT, with OD measured at 405 nm using an ELISA reader (Thermo Fisher Scientific Co, USA). The tissue lysis steps in this test were similar to those for measuring MDA, and the catalase enzyme activity was calculated using the following formula.

Catalase activity (v/ml) = (OD blank - OD sample) × 271 × (1/60 * sample volume).

2.4. Expression of inflammatory and apoptotic genes *TNF-α*, *IL-6*, *Bcl-2* and *Bax*

This study utilized relative quantification through real-time PCR to analyze gene expression. Initially, RNA was extracted using the Total RNA Extraction Kit (Cinnagen Co, Iran). Following the extraction, the quantity and concentration of the RNA were assessed, and complementary DNA (cDNA) was synthesized according to the protocol provided by the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The thermocycler temperature cycles were conducted as per the kit instructions, and the resulting reaction products were stored at -20°C. The quality and uniformity of the cDNA samples were evaluated using a NanoDrop One C spectrophotometer (Thermo Scientific, USA). For the real-time PCR analysis, the extracted cDNA, SYBR Green Master Mix, and specifically designed primers for *TNF-α*, *IL-6*, *Bcl-2*, and *Bax* genes were utilized. SYBR Green is a DNA-binding dye that intercalates within the minor groove of DNA. The specificity of the real-time PCR results was confirmed by analyzing the melting curve, which helps detect non-specific amplification products.

The PCR was performed on a Rotor-Gene Q system (QIAGEN Co, Germany) with a total reaction volume of 20 µl, which included 8 µl of DEPC-treated water, 10 µl of 2X SYBR Green Master Mix, 1 µl of synthesized cDNA, and 1 µl of primers. The cycling conditions were as follows: one cycle at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 20 seconds, 52 °C for 15 seconds, and 72 °C for 20 seconds, with a final step of gradual heating from 55 °C to 94 °C in 1 °C increments for 1 second each. The sequences of the forward and reverse primers for *TNF-α*, *IL-6*, *Bcl-2*, and *Bax* are presented in Table 1. Finally, to investigate the decrease or increase in the expression of the studied genes, *GAPDH* was used as a reference gene.

The PCR was performed on a Rotor-Gene Q system (QIAGEN Co, Germany) with a total reaction volume of 20 µl, which included 8 µl of DEPC-treated water, 10 µl of 2X SYBR Green Master Mix, 1 µl of synthesized cDNA, and 1 µl of primers. The cycling conditions were as follows: one cycle at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 20 seconds, 52 °C for 15 seconds, and 72 °C for 20 seconds, with a final step of gradual heating from 55 °C to 94 °C in 1 °C increments for 1 second each. The sequences of the forward and reverse primers for *TNF-α*, *IL-6*, *Bcl-2*, and *Bax* are presented in Table 1. Finally, to investigate the decrease or increase in the expression of the studied genes, *GAPDH* was used as a reference gene.

The PCR was performed on a Rotor-Gene Q system (QIAGEN Co, Germany) with a total reaction volume of 20 µl, which included 8 µl of DEPC-treated water, 10 µl of 2X SYBR Green Master Mix, 1 µl of synthesized cDNA, and 1 µl of primers. The cycling conditions were as follows: one cycle at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 20 seconds, 52 °C for 15 seconds, and 72 °C for 20 seconds, with a final step of gradual heating from 55 °C to 94 °C in 1 °C increments for 1 second each. The sequences of the forward and reverse primers for *TNF-α*, *IL-6*, *Bcl-2*, and *Bax* are presented in Table 1. Finally, to investigate the decrease or increase in the expression of the studied genes, *GAPDH* was used as a reference gene.

2.5. Statistical Analysis of Data

Statistical analysis for this study was performed using SPSS version 16, and the results were examined through one-way ANOVA. The data are presented as mean ± standard deviation (SD), with a significance level set at P < 0.05. The normal distribution of the antioxidant enzymes and urinary serum factors data was done by the Kolmogorov-Smirnov test (P>0.05). Examination of pathological sections of kidney tissues was done by a semi-quantitative scale. The severity of kidney damage was evaluated by the method provided by Gulgun et al. (34) The semi-quantitative grading is included as below; the absence of histological changes (grade zero), minor histological changes (grade one), moderate histological changes (grade two), severe histological changes (grade three), Assessed blindly by two pathologists. Differences in the expression of target genes between control and treated samples were analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test using GraphPad Prism version 6.0. Real-time PCR data analysis was conducted based on the comparison of cycle thresholds (Ct). In this study, the differences in Ct values obtained from the treated samples (probiotic-treated cells) and control samples were calculated, and the ratio of the target gene to the reference gene was determined using the formula $2^{-\Delta\Delta Ct}$. The calculation formula is as follows.

$$\Delta Ct = Ct_{\text{test sample}} - Ct_{\text{reference control sample}}$$

$$\text{Relative expression} = 2^{-\Delta\Delta Ct}$$

Table 1. The sequence of primers used in Real-time PCR.

Primers	Sequence	Tm	Reference
<i>Bax</i> Forward	TCCCCCGAGAGGTCTTTT3	54 °C	(35)
<i>Bax</i> Reverse	CGGCCCCAGTTGAAGTTG3	54 °C	
<i>Bcl-2</i> Forward	TTGGCCCCCGTTGCTT	55 °C	(35)
<i>Bcl-2</i> Reverse	CGGTTATCGTACCCCGTTCTC	55 °C	
<i>GAPDH</i> Forward	GAAGGTGAAGGTCGGAGTC	54 °C	(36)
<i>GAPDH</i> Reverse	GAAGATGGTGATGGGATTTC	54 °C	
<i>TNF-α</i> Forward	CCAGGAGAAAGTCAGCCTCCT	56 °C	(37)
<i>TNF-α</i> Reverse	TCATACCAGGGCTTGAGCTCA	56 °C	
<i>IL-6</i> Forward	GTCTTGCCATGCTAAAGGACG	55 °C	(38)
<i>IL-6</i> Reverse	TGGCTTGTTCTCACTACTCTC	55 °C	

3. Results

3.1. Results of the Probiotic Treatment on Biochemical Factors

As shown in Figure 1, the mean serum urea level in the control group was 32.2 ± 6.5 mg/dL, which significantly increased to 88.2 ± 7.3 mg/dL in the HgCl₂-treated group ($P < 0.0001$). Co-treatment with probiotic reduced urea levels to 67.6 ± 5.6 mg/dL. Serum creatinine also increased from 0.95 ± 0.191 mg/dL in the control group to 2.8 ± 0.593 mg/dL in the toxin group ($P < 0.0001$), and decreased to 1.77 ± 0.33 mg/dL in the probiotic+toxin group. The differences were statistically significant (ANOVA, Tukey post hoc).

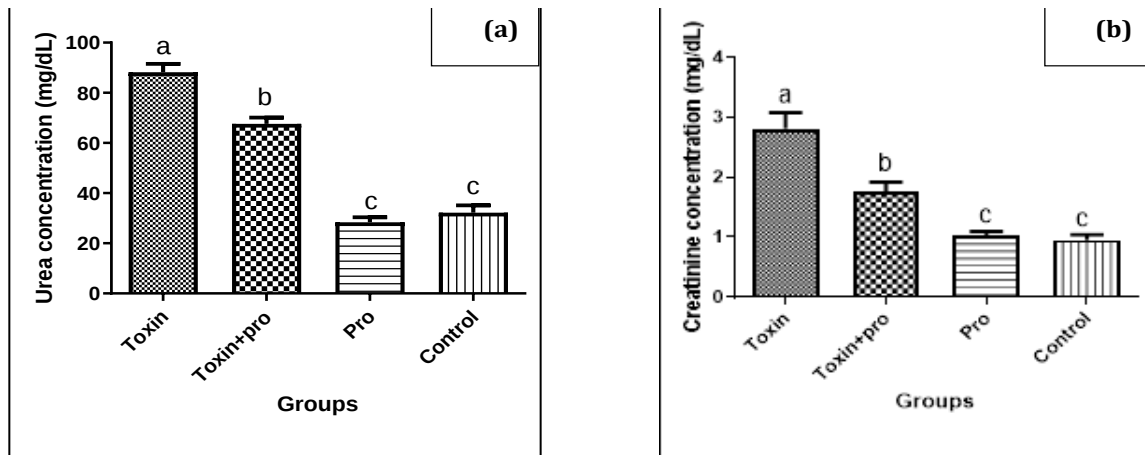


Fig1. Diagram of (a) urea and (b) creatinine changes among study groups after treatment

The observed changes among the groups were completely significant, with a P value < 0.0001 . A statistically significant level was considered equivalent to a P value ≤ 0.05 .

3.2. Results of the Probiotic Treatment on Antioxidant Factors

According to the data obtained from the Tukey post-hoc test, in the control group, SOD activity was 51.6 ± 5.6 IU/mL, which decreased to 35.7 ± 1.7 IU/mL in the HgCl_2 group and increased to 42.8 ± 3.2 IU/mL in the probiotic+toxin group. CAT activity dropped from 8.94 ± 2.36 mU/mL (control) to 0.71 ± 0.42 mU/mL (toxin) and improved to 3.1 ± 1.97 mU/mL with probiotic co-treatment. MDA levels rose from 6.08 ± 1.2 μM in the control group to 16.75 ± 1.13 μM in the toxin group and decreased to 9.42 ± 0.65 μM in the probiotic+toxin group. CAT and MDA changes were statistically significant ($P < 0.0001$), while SOD differences between toxin and probiotic+toxin groups were not statistically significant (Figure 2).

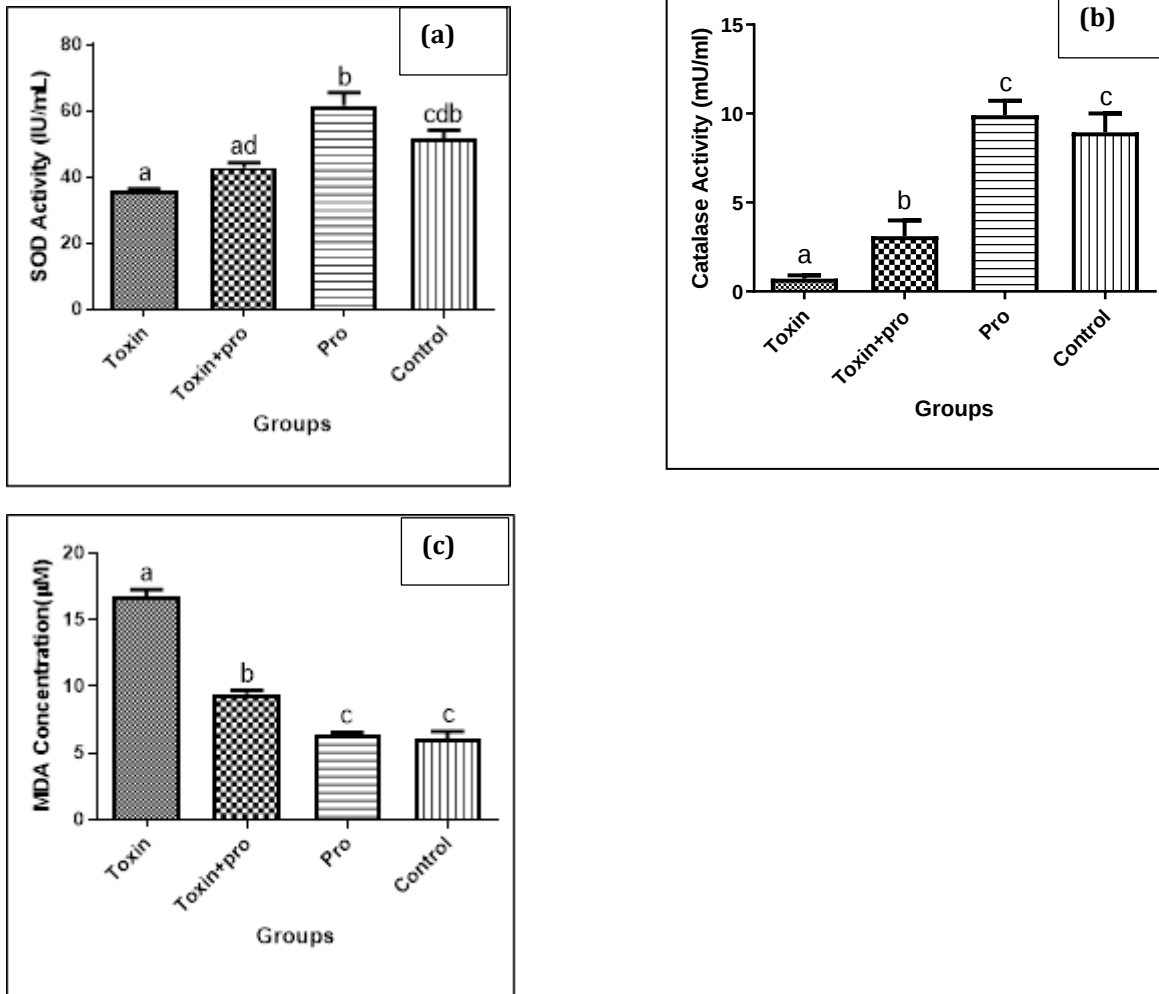


Fig 2. Diagram of (a) SOD, (b) CAT, and (c) MDA changes among study groups after treatment

The observed changes among the groups were completely significant, with P value < 0.0001 . A statistically significant level was considered equivalent to a P value ≤ 0.05 .

3.3. Changes in Gene Expression Levels in Different Treatment Groups

Real-Time PCR results indicated significant upregulation of IL-6, TNF- α , and BAX genes in the toxin group compared to the control group ($P < 0.05$). Co-treatment with probiotic significantly reduced their expression compared to the toxin group ($P < 0.05$). BCL2 expression, which was downregulated in the toxin group, significantly increased in the probiotic+toxin group ($P = 0.0052$). Gene expression changes were calculated using the $2^{-\Delta\Delta C_t}$ method and are reported as mean $\pm$ SE.

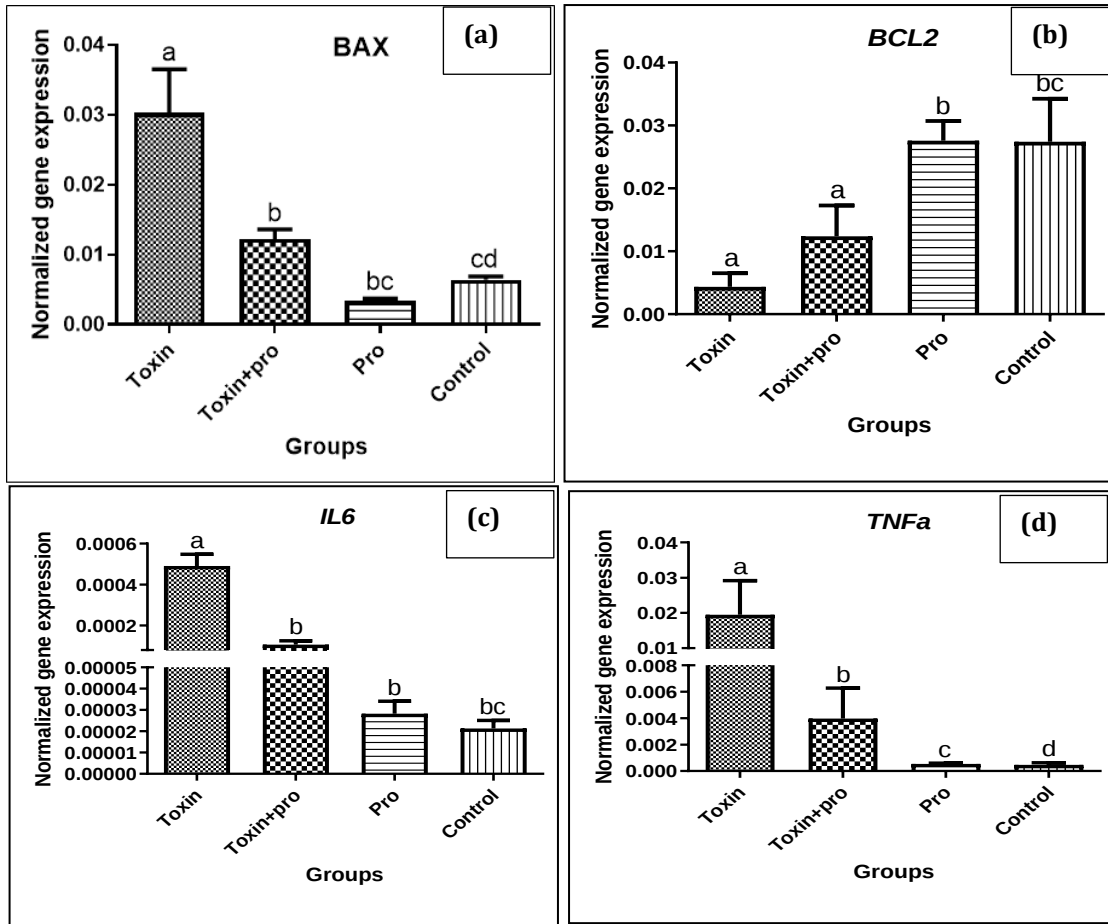


Fig 3. Diagram of (a) *BAX*, (b) *BCL2*, (c) *IL-6*, and (d) *TNFα* gene expression changes among study groups after treatment

*A statistically significant level was considered equivalent to a P value ≤ 0.05 .

3.4. Kidney Tissue Pathology

The results of the pathological examination and microscopic analysis of kidney tissue biopsies are presented in Figure 4. The mean glomerular size in the control group was $65.4 \pm 2.7 \mu\text{m}$, which significantly decreased to $13.4 \pm 2.7 \mu\text{m}$ in the HgCl_2 -treated group ($P < 0.0001$). In the probiotic+toxin group, the glomerular size significantly increased to $35.2 \pm 4.8 \mu\text{m}$, indicating the protective effect of probiotic administration on renal tissue architecture (Figure 5).

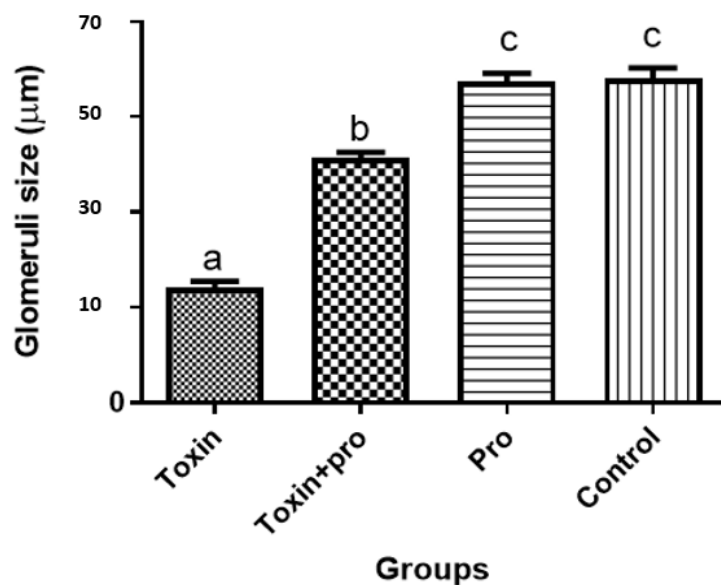


Fig 4. Diagram of changes in the size of renal glomeruli among the study groups in micrometers.

*A statistically significant level was considered equivalent to a P value ≤ 0.05 .

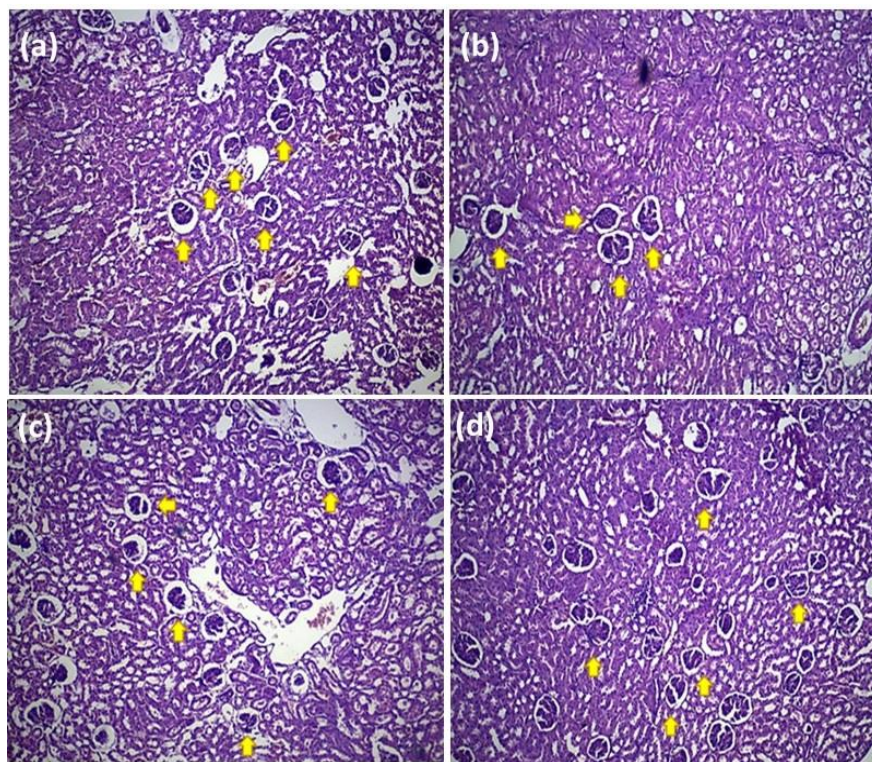


Fig 5. The size of renal glomeruli in four groups of (a) control, (b) toxin, (c) probiotics, (d) toxin + probiotics at 200 μm magnification

4. Discussion

According to research by Badr et al. (2023), nephrotoxic agents induce oxidative stress and inflammation, leading to renal injury and altered kidney function markers (39). Probiotics have been shown to have protective effects on kidney function, possibly through mechanisms such as modulation of gut microbiota, reduction of inflammation, and enhancement of the intestinal barrier. Zhu et al. (2021) demonstrated that certain probiotic strains could alleviate kidney damage by inhibiting inflammatory pathways and enhancing antioxidant activity (40). In this study, findings regarding the levels of urea and creatinine in the toxin group compared to other groups align with existing literature on the nephrotoxic effects of various substances. The observation that the probiotic group did not show significant changes compared to the control group, but the toxin group combined with probiotics exhibited moderately reduced levels of urea and creatinine compared to the toxin group alone, suggests that probiotic administration may have a partial protective effect on renal function. While the levels remained elevated compared to the control, the co-treatment mitigated the extent of biochemical disruption induced by HgCl₂ exposure.

Many studies document how exposure to nephrotoxic agents (like heavy metals or certain drugs) leads to elevated levels of urea and creatinine due to impaired renal function (41-43). The significant increase in urea and creatinine levels in the toxin group is consistent with findings from Ali et al. (2019), who reported similar results in their study on renal toxicity induced by chemical agents. Their research emphasized the importance of monitoring these biomarkers in toxicological assessments (44). The lack of significant changes in the probiotic group compared to the control in this research is also reported in studies where probiotics did not show immediate effects on kidney function under normal conditions. Hasain et al. (2022) noted that while probiotics may have long-term benefits for kidney health, acute changes in biomarkers might not be immediately evident (45). The finding that the toxin group combined with probiotics exhibited a significant increase in urea and creatinine levels is intriguing. It suggests that while probiotics might have protective effects, their presence in a toxic environment could potentially exacerbate the biochemical markers due to competing metabolic pathways or interactions. Baralić et al. (2023) explored the adverse effects of probiotics in certain contexts, indicating that the efficacy of probiotics can be context-dependent, especially in the presence of toxins (46). Conversely, Elazab et al. (2021) demonstrated that administration of specific probiotics significantly lowered serum creatinine and urea levels in rats subjected to nephrotoxic agents (47), which is not in agreement with this study. The researchers suggested that probiotics could mitigate oxidative stress and inflammation, leading to improved kidney function markers. In a randomized controlled trial, Rahman and Meizel (2024) reported that patients with mild kidney impairment who received probiotics showed no significant changes in serum urea and creatinine compared to a control group (48). This aligns with findings obtained in this investigation that probiotics may not always lead to measurable improvements in kidney function, especially in less severe cases. Dai et al. (2022)

examined various strains of probiotics and their effects on kidney function in a diabetic nephropathy model. While some strains showed protective effects (reducing serum creatinine), others did not show significant changes (19). This indicates that the effects of probiotics can be strain-specific and context-dependent.

Results highlight the protective role of probiotics in maintaining SOD activity as no significant difference was observed between the probiotic + toxin and control groups, but also suggest limitations in their ability to counteract the effects of certain toxins on CAT activity, as CAT remained significantly lower even in the probiotic + toxin group. SOD is an essential antioxidant enzyme that plays a critical role in the dismutation of superoxide radicals into hydrogen peroxide and oxygen (49). Research by Zhao et al. (2020) indicated that probiotics can enhance the activity of SOD, thereby reducing oxidative stress in various biological contexts (50). The current research report that SOD activity remains stable in the probiotic + toxin group compared to the control group suggests that probiotics may help maintain cellular antioxidant defenses even in the presence of toxins, possibly through the modulation of gut microbiota and reduction of systemic inflammation. The result is somewhat aligned with the findings of Khan et al. (2024), who reported that some probiotic strains had no significant effect on SOD activity under normal physiological conditions (51). CAT is crucial for the decomposition of hydrogen peroxide, a byproduct of SOD activity (49). The significant decrease in CAT activity in the probiotic + toxin group compared to the control group, as noted in this study, suggests that while probiotics may stabilize SOD under toxic conditions, they may not be sufficient to prevent the oxidative damage reflected in CAT activity. This finding aligns with Varada et al. (2022), who reported that certain toxins could overwhelm the antioxidant capacity of both SOD and CAT, thereby impairing their activity despite the presence of probiotics (52). In contrast, Wang et al. (2022) found that certain probiotics could enhance CAT activity in stressed conditions, indicating that the efficacy of probiotics may vary depending on the strain and the nature of the toxin used (53). MDA is also a marker of lipid peroxidation and is often used as an indicator of oxidative stress (54). In this study, MDA levels were significantly elevated in the toxin group but partially reduced in the probiotic + toxin group, while the probiotic-only group showed values similar to the control, suggesting a protective effect of probiotics against lipid peroxidation. This is consistent with findings from Sajjad et al. (2024), who observed that probiotics could lower MDA levels in models of oxidative stress, indicating their role in reducing lipid damage (55). Furthermore, this finding resonates with studies like Ghosh et al. (2024), who reported that probiotics can effectively reduce lipid peroxidation in various toxin-induced injury models (56).

BAX is a pro-apoptotic gene that, when overexpressed, promotes apoptosis, while *BCL2* is an anti-apoptotic gene that helps to inhibit cell death (57). The current investigation shows a significant decrease in *BAX* expression with *L. casei* treatment in the probiotic + toxin group, consistent with existing literature indicating that various toxins can induce apoptosis through mitochondrial pathways. For instance, Gur et al. (2022)

, which showed that specific probiotic strains could inhibit apoptosis in liver cells exposed to toxins (59). Patel et al. (2022), who reported that probiotics could downregulate *BAX* expression in models of oxidative stress (60). Besides, the present observation that probiotics significantly increased *BCL2* expression only in the presence of toxins contrasts with findings from Li et al. (2021), who reported that certain probiotics could enhance *BCL2* expression independently of toxin exposure, suggesting that the effects may vary by strain and context (61). Both *IL-6* and *TNF-α* are pro-inflammatory cytokines that are often elevated in response to stress and injury (62). Current results showing significant increases in these genes in the toxin group are consistent with previous studies, such as Khurshaid et al. (2023), which demonstrated that toxins induce inflammation by upregulating *IL-6* and *TNF-α* expression in renal tissues (63). The significant reduction of *IL-6* and *TNF-α* expression in the probiotic + toxin group suggests that *L. casei* may exert anti-inflammatory effects, potentially through modulation of gut microbiota and systemic inflammatory responses. This is supported by Das et al. (2022), who found that probiotics could reduce the expression of inflammatory cytokines in various models (64). However, in this research, probiotics alone did not significantly affect these cytokines, in line with Zhou et al. (2022), who found that while probiotics have protective effects, their efficacy in reducing inflammation may depend on the presence of stressors (65).

5. Conclusion

In conclusion, the findings of this study highlight the protective role of *L. casei* against kidney damage induced by HgCl₂. While the probiotic treatment did not significantly alter biochemical markers of kidney function or antioxidant enzyme activity in isolation, it effectively mitigated the negative effects of toxin exposure on these parameters. Additionally, the administration of *L. casei* significantly reduced the expression of pro-apoptotic and inflammatory genes, suggesting its potential to modulate oxidative stress and inflammation. Pathological assessments further confirmed the restoration of renal glomerular structure, emphasizing the therapeutic potential of probiotics in safeguarding kidney health against toxic insults. Overall, these results support the incorporation of probiotics as a viable strategy for renal protection in toxic exposure scenarios.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Acknowledgments

The authors would like to acknowledge that no external assistance or support was required for this study.

Ethics approval

All animal procedures were approved by the Committee of Animal Ethics of the (R.IAU.PIAU.REC.1401.014). All methods are reported by ARRIVE guidelines (<https://arriveguidelines.org>).

References

1. Zafar A, Javed S, Akram N, Naqvi SAR. Health Risks of Mercury. Mercury Toxicity Mitigation: Sustainable Nexus Approach: Springer; 2024. p. 67-92.
2. Bansal H. Heavy metal toxicity: A comprehensive review of forms, exposure routes, toxicokinetics, and effects on infants. International Journal of Medical Toxicology & Legal Medicine. 2023;26(1and2):13-24.
3. Rahmati F, Sethi D, Shu W, Lajayer BA, Mosaferi M, Thomson A, et al. Advances in microbial exoenzymes bioengineering for improvement of bioplastics degradation. Chemosphere. 2024;355:141749.
4. Imenez Silva PH, Mohebbi N. Kidney metabolism and acid-base control: back to the basics. Pflügers Archiv-European Journal of Physiology. 2022;474(8):919-34.
5. Singh S, Trivedi SP, Kumar M. Prolonged exposure to mercuric chloride induces oxidative stress-mediated nephrotoxicity in freshwater food fish *Channa punctatus*. Environmental Science and Pollution Research. 2024;1:15.
6. Mohamed SH, El-Kady DS, Elmegeed GA, El-kassaby MI, Farrag A-RH, Abdelhalim MM, et al. The efficacy of novel heterocyclic activators against mercuric chloride-induced acute renal failure in rats. Journal of Applied Pharmaceutical Science. 2023;13(4):070-83.
7. Patial V, Dadhich G, Kumar R. Insights from Proteomics in Kidney Disease Diagnosis and Various In Vitro and In Vivo Experimental Models. Sustainable Agriculture Reviews 57: Animal Biotechnology for Livestock Production 2: Springer; 2022. p. 27-69.
8. Wei X, Hou Y, Long M, Jiang L, Du Y. Molecular mechanisms underlying the role of hypoxia-inducible factor-1α in metabolic reprogramming in renal fibrosis. Frontiers in endocrinology. 2022;13:927329.
9. Han B, Lv Z, Han X, Li S, Han B, Yang Q, et al. Harmful effects of inorganic mercury exposure on kidney cells: mitochondrial dynamics disorder and excessive oxidative stress. Biological Trace Element Research. 2022;200(4):1591-7.
10. Rahmati F, Asgari Lajayer B, Shadfar N, van Bodegom PM, van Hullebusch ED. A review on biotechnological approaches applied for marine hydrocarbon spills remediation. Microorganisms. 2022;10(7):1289.
11. Mumtaz F, Farag BM, Farahat MA, Farouk FA, Aarif MY, Eltresy MH, et al. Leek (*Allium ampeloprasum* var. *kurrat*) aqueous extract loaded on selenium nanoparticles protects against testis and brain injury induced by mercuric chloride in rats. Journal of the Science of Food and Agriculture. 2024.
12. Sheri V, Kumar M, Jaconis S, Zhang B. Antioxidant defense in cotton under environmental stresses: unraveling the crucial role of a universal defense regulator for enhanced cotton sustainability. Plant Physiology and Biochemistry. 2023;108:141.

13. Coluk Y, Peker EGG, Yildirmak S, Keskin A, Yildirim G. Exploring the protective role of green tea extract against cardiovascular alterations induced by chronic REM sleep deprivation via modulation of inflammation and oxidative stress. *BMC Complementary Medicine and Therapies*. 2024;24(1):351.
14. Wang J, Yin Y, Zhang Q, Deng X, Miao Z, Xu S. HgCl₂ exposure mediates pyroptosis of HD11 cells and promotes M1 polarization and the release of inflammatory factors through ROS/Nrf2/NLRP3. *Ecotoxicology and environmental safety*. 2024;269:115779.
15. Ramadan SS, El Zaiat FA, Habashy EA, Montaser MM, Hassan HE, Tharwat SS, et al. Coenzyme Q10-Loaded Albumin Nanoparticles Protect against Redox Imbalance and Inflammatory, Apoptotic, and Histopathological Alterations in Mercuric Chloride-Induced Hepatorenal Toxicity in Rats. *Biomedicine*. 2023;11(11):3054.
16. Maria Francis Y, Karunakaran B, Ashfaq F, Yahia Qattan M, Ahmad I, Alkhatami AG, et al. Mercuric chloride induced nephrotoxicity: Ameliorative effect of Carica papaya leaves confirmed by histopathology, immunohistochemistry, and gene expression studies. *ACS omega*. 2023;8(24):21696-708.
17. Rahmati F. Characterization of Lactobacillus, Bacillus and Saccharomyces isolated from Iranian traditional dairy products for potential sources of starter cultures. *AIMS microbiology*. 2017;3(4):815.
18. Rahmati F. Identification and characterization of Lactococcus starter strains in milk-based traditional fermented products in the region of Iran. *AIMS Agriculture & Food*. 2018;3(1).
19. Dai Y, Quan J, Xiong L, Luo Y, Yi B. Probiotics improve renal function, glucose, lipids, inflammation and oxidative stress in diabetic kidney disease: a systematic review and meta-analysis. *Renal failure*. 2022;44(1):862-80.
20. Rahmati F. Microencapsulation of Lactobacillus acidophilus and Lactobacillus plantarum in Eudragit S100 and alginate chitosan under gastrointestinal and normal conditions. *Applied Nanoscience*. 2020;10(2):391-9.
21. Feng T, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut microbes*. 2020;12(1):1801944.
22. Blazheva D, Mihaylova D, Averina O, Slavchev A, Brazkova M, Poluektova E, et al. Antioxidant potential of probiotics and postbiotics: a biotechnological approach to improving their stability. *Russian Journal of Genetics*. 2022;58(9):1036-50.
23. Rahmati F. Impact of microencapsulation on two probiotic strains in alginate chitosan and Eudragit S100 under gastrointestinal and normal conditions. *The Open Biotechnology Journal*. 2019;13(1).
24. Zhao M, Li Y, Wang Z. Mercury and mercury-containing preparations: history of use, clinical applications, pharmacology, toxicology, and pharmacokinetics in traditional Chinese medicine. *Frontiers in Pharmacology*. 2022;13:807807.
25. Zhou Y, Vaidya VS, Brown RP, Zhang J, Rosenzweig BA, Thompson KL, et al. Comparison of kidney injury molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury, and chromium. *Toxicological sciences*. 2008;101(1):159-70.
26. Van Pelt LF. Ketamine and xylazine for surgical anesthesia in rats. *Journal of the American Veterinary Medical Association*. 1977;61(9):1171.
27. Patton CJ, Crouch S. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Analytical chemistry*. 1977;49(3):464-9.
28. Levy GB. Determination of sodium with ion-selective electrodes. *Clinical chemistry*. 1981;27(8):1435-8.
29. Orndorff W, Patel M. ORTHO-CRESOL-TETRACHLOROPHTHALEIN, SOME OF ITS DERIVATIVES, AND ISO-ORTHO-CRESOL-TETRACHLOROPHTHALEIN. *Journal of the American Chemical Society*. 1925;47(3):863-7.
30. Zhao Y, Yang X, Lu W, Liao H, Liao F. Uricase based methods for determination of uric acid in serum. *Microchimica Acta*. 2009;164:1-6.
31. Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Analytical biochemistry*. 1986;154(2):536-41.
32. De K, Roy K, Saha A, Sengupta C. LIPID PEROXIDATION USING (COMMON LABORATORY MARKERS. *Acta poloniae pharmaceutica*. 2004;61(1):77-90.
33. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem*. 1952;195(1):133-40.
34. Gulgun M, Erdem O, Oztas E, Kesik V, Balamtekin N, Vurucu S, et al. Proanthocyanidin prevents methotrexate-induced intestinal damage and oxidative stress. *Experimental and Toxicologic Pathology*. 2010;62(2):109-15.
35. Ekundina VO, Omon EA. Genomic analysis of BAX and Bcl-2 gene mutations in human papilloma virus-associated squamous cell carcinoma of the cervix. *Surgical and Experimental Pathology*. 2024;7(1):11.
36. Viader-Salvadó JM, Pentón-Piña N, Robainas-del-Pino Y, Fuentes-Garibay JA, Guerrero-Olazarán M. Effect of AOX1 and GAP transcriptional terminators on transcript levels of both the heterologous and the GAPDH genes and the extracellular Yp/x in GAP promoter-based Komagataella phaffii strains. *PeerJ*. 2024;12:e18181.
37. Shobeiri SS, Dashti M, Pordel S, Rezaee M, Haghnava N, Moghadam M, et al. Topical anti-TNF- α ssDNA aptamer decreased the imiquimod induced psoriatic inflammation in BALB/c mice. *Cytokine*. 2023;172:156406.

38. Liu M, Li H, Zhang H, Zhou H, Jiao T, Feng M, et al. RBMS1 promotes gastric cancer metastasis through autocrine IL-6/JAK2/STAT3 signaling. *Cell Death & Disease*. 2022;13(3):287.
39. Badr AM, Al-Kharashi LA, Attia H, Alshehri S, Alajami HN, Ali RA, et al. TLR4/inflammasomes cross-talk and pyroptosis contribute to N-Acetyl cysteine and chlorogenic acid protection against cisplatin-induced nephrotoxicity. *Pharmaceuticals*. 2023;16(3):337.
40. Zhu H, Cao C, Wu Z, Zhang H, Sun Z, Wang M, et al. The probiotic *L. casei* Zhang slows the progression of acute and chronic kidney disease. *Cell metabolism*. 2021;33(10):1926-42. e8.
41. Mishra M, Nichols L, Dave AA, Pittman EH, Cheek JP, Caroland AJ, et al. Molecular mechanisms of cellular injury and role of toxic heavy metals in chronic kidney disease. *International Journal of Molecular Sciences*. 2022;23(19):11105.
42. Okpogba A, Ogbodo E, Ezeugwunne I, Analike R, Amah A, Ikimi C, et al. Assessment of the renal function status in occupationally exposed people working in metal fabricating factory in Nnewi. *International Journal of Pharmaceutical Chemistry and Analysis*. 2021;7(1):54-60.
43. Cabral M, Garçon G, Touré A, Bah F, Dewaele D, Bouhsina S, et al. Renal impairment assessment on adults living nearby a landfill: Early kidney dysfunction biomarkers linked to the environmental exposure to heavy metals. *Toxicology Reports*. 2021;8:386-94.
44. Ali S, Hussain S, Khan R, Mumtaz S, Ashraf N, Andleeb S, et al. Renal toxicity of heavy metals (cadmium and mercury) and their amelioration with ascorbic acid in rabbits. *Environmental Science and Pollution Research*. 2019;26:3909-20.
45. Hasain Z, Raja Ali RA, Ahmad HF, Abdul Rauf UF, Oon SF, Mokhtar NM. The roles of probiotics in the gut microbiota composition and metabolic outcomes in asymptomatic post-gestational diabetes women: a randomized controlled trial. *Nutrients*. 2022;14(18):3878.
46. Baralić K, Živančević K, Bozic D, Đukić-Čosić D. Probiotic cultures as a potential protective strategy against the toxicity of environmentally relevant chemicals: State-of-the-art knowledge. *Food and Chemical Toxicology*. 2023;172:113582.
47. Elazab ST, Elshater NS, Kishaway AT, Ei-Emam HA. Cinnamon extract and probiotic supplementation alleviate copper-induced nephrotoxicity via modulating oxidative stress, inflammation, and apoptosis in broiler chickens. *Animals*. 2021;11(6):1609.
48. Rahman WK, Rabaa IS, Meizel MM. Protective Effect of Probiotic Against Progression of Chronic Kidney Disease: A Randomized Clinical Study. *Medical Journal of Babylon*. 2024;21(3):560-6.
49. Rahmati F, Hosseini SS, Mahuti Safai S, Asgari Lajayer B, Hatami M. New insights into the role of nanotechnology in microbial food safety. *3 Biotech*. 2020;10:1-15.
50. Zhao J, Yu L, Zhai Q, Tian F, Zhang H, Chen W. Effects of probiotic administration on hepatic antioxidative parameters depending on oxidative stress models: A meta-analysis of animal experiments. *Journal of Functional Foods*. 2020;71:103936.
51. Khan AN, Ghazanfar S, Hassan MN, Ahmad A, Khan N, Khalid S, et al. Probiotic potential of *Lactobacillus agilis* against oxidative, inflammatory and diabetic stresses. *Journal of King Saud University-Science*. 2024;36(5):103144.
52. Varada VV, Kumar S, Tyagi N, Tyagi AK. Effects of compound lyophilized probiotics on selected faecal microbiota, immune response, and antioxidant status in newborn buffalo calves. *Current Research in Biotechnology*. 2022;4:493-502.
53. Wang W, Dong H, Chang X, Chen Q, Wang L, Chen S, et al. *Bifidobacterium lactis* and *Lactobacillus plantarum* enhance immune function and antioxidant capacity in cats through modulation of the gut microbiota. *Antioxidants*. 2024;13(7):764.
54. Carmo de Carvalho e Martins Md, Martins, da Silva Santos Oliveira AS, da Silva LAA, Primo MGS, de Carvalho Lira VB. Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. *Biomarkers in Nutrition: Springer*; 2022. p. 1-25.
55. Sajjad A, Ali S, Mumtaz S, Summer M, Farooq MA, Hassan A. Chemoprotective and immunomodulatory potential of *Lactobacillus reuteri* against cadmium chloride-induced breast cancer in mice. *Journal of Infection and Chemotherapy*. 2024.
56. Ghosh S, Ghosh AJ, Islam R, Sarkar S, Saha T. *Lactobacillus plantarum* KAD protects against high-fat diet-induced hepatic complications in Swiss albino mice: Role of inflammation and gut integrity. *PloS one*. 2024;19(11):e0313548.
57. Singh L, Pushker N, Saini N, Sen S, Sharma A, Bakhshi S, et al. Expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins in human retinoblastoma. *Clinical & experimental ophthalmology*. 2015;43(3):259-67.
58. Gur C, Kandemir FM, Caglayan C, Satıcı E. Chemopreventive effects of hesperidin against paclitaxel-induced hepatotoxicity and nephrotoxicity via amendment of Nrf2/HO-1 and caspase-3/Bax/Bcl-2 signaling pathways. *Chemico-biological interactions*. 2022;365:110073.
59. Ullah A, Mu J, Wang F, Chan MWH, Yin X, Liao Y, et al. Biogenic selenium nanoparticles and their anticancer effects pertaining to probiotic bacteria—A Review. *Antioxidants*. 2022;11(10):1916.
60. Patel C, Shahgond L, Acharya S, Boddur SH, Ranch K. Protective effect of probiotics and ascorbic acid on bile duct ligation-induced chronic hepatic encephalopathy in rats. *Research in Pharmaceutical Sciences*. 2022;17(4):445-56.

61. Li J, Li Q, Gao N, Wang Z, Li F, Li J, et al. Exopolysaccharides produced by *Lactobacillus rhamnosus* GG alleviate hydrogen peroxide-induced intestinal oxidative damage and apoptosis through the Keap1/Nrf2 and Bax/Bcl-2 pathways in vitro. *Food & Function*. 2021;12(20):9632-41.
62. Li R, Ye JJ, Gan L, Zhang M, Sun D, Li Y, et al. Traumatic inflammatory response: pathophysiological role and clinical value of cytokines. *European Journal of Trauma and Emergency Surgery*. 2024;50(4):1313-30.
63. Khurshaid I, Ilyas S, Zahra N, Ahmad S, Aziz T, Al-Asmari F, et al. Isolation, preparation and investigation of leaf extracts of *Aloe barbadensis* for its remedial effects on tumor necrosis factor alpha (TNF- α) and interleukin (IL-6) by in vivo and in silico approaches in experimental rats. *Acta Biochimica Polonica*. 2023;70(4):927-33.
64. Das TK, Pradhan S, Chakrabarti S, Mondal KC, Ghosh K. Current status of probiotic and related health benefits. *Applied Food Research*. 2022;2(2):100185.
65. Zhou B, Ba T, Wang L, Gao Y, He Q, Yan Z, et al. Combination of sodium butyrate and probiotics ameliorates severe burn-induced intestinal injury by inhibiting oxidative stress and inflammatory response. *Burns*. 2022;48.2022-1213(5)