Received: 21 August 2019 | Revised: 20 January 2020 | Accepted: 14 February 2020

DOI: 10.1002/jbt.22481

WILEY

Role of α-tocopherol and *Lactobacillus plantarum* in the alleviation of mercuric chloride-induced testicular atrophy in rat's model: Implication of molecular mechanisms

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Funding information
Deanship of Scientific Research at King Saud University, Grant/Award Number: (RG-1440-017).

Abstract

The present work was aimed to evaluate the protective effects of alpha-tocopherol (α-toco) and/or *Lactobacillus plantarum* (LCB) against testicular atrophy induced by mercuric chloride (MCH). Rats were injected with 5 mg/kg MCH for 5 days consecutively, then treated with 100 mg/kg α-toco and 6×10^{10} CFU 1.8701/kg LCB alone or together for 3 weeks. The MCH elevated serum TNF-α, IL- 6, caspase-3, and testicular malondialdehyde. However, serum testosterone, dehydroepiandrosterone, testicular messenger RNA of a steroidogenic acute regulatory protein, 17-β-hydroxysteroid dehydrogenase, 3β-hydroxysteroid dehydrogenase, glutathione level, and superoxide dismutase activity were decreased. Protein expression of Nrf2 was downregulated whereas that of Bax and DNA fragmentation was upregulated in the testicular tissues. Treatment with α-toco and LCB ameliorated the deviated biochemical parameters and improved tissue injury. It was concluded that the combination of LCB and α-toco achieved promising results in the amelioration of MCH-induced testicular atrophy. Nrf2, Bax expressions, and DNA fragmentation are involved in the testicular atrophy induced by MCH.

KEYWORDS

17β-HSD, 3β-HSD, gene expression, *Lactobacillus plantarum*, mercury, rats, STAR, testes, α-tocopherol

1 | INTRODUCTION

Mercury is used in several drugs, whitening creams, disinfectants, sanitizers, preservatives in makeup, toothpaste, solutions for eye lens, vaccines and immune-therapy solutions, herbicides, fungicides and an amalgam of teeth.[1] Mercury toxicity is largely attributed to its ability to induce oxidative stress by enhancement of the generation of reactive oxygen species (ROS) and disturbing the activities of several enzymes including those having antioxidant effects and that containing the thiol group.[2]

The usual therapeutic intervention for metal toxicity is chelation therapy. Metal-detoxifying agents work by forming less toxic complexes with the harmful metal ions that facilitate their excretion. The usually used chelating agents are accompanied by side effects like liver and renal toxicities, critical loss of metals, as well as dermal reactions.[3] Thus, the emergence of novel metal-detoxifying agents is necessary.

Alpha-tocopherol (α-toco) is critical for keeping the cellular membrane function, flexibility, and integrity. α-Toco protects the body by detoxifying harmful compounds and removing reactive nitrogen species.[4] Besides this, α-toco improves normal cellular division and immunity, controls the coagulation speed of the blood and protects nerve tissues.[4] Agarwal et al[5] found that mercury causes oxidative damage and metallothionein messenger RNA (mRNA) expression as well as histological modifications in tissue and accumulation of mercury in several

organs. In addition, both pretreatment and posttreatment with α-toco provide complete protection of the liver from mercury toxicity. Probiotics represent a class of biomedical products with high efficacy and limited side effects making them suitable for therapeutic intervention.

In vitro, *Lactobacillus Plantarum* (LCB) was able to bind heavy metals like cadmium, lead, and aflatoxin B1 as well.[6,7] Moreover, LCB is identified as an antioxidant in human subjects.[8,9] Accordingly, LCB has been established as a probiotic for the mitigation and management of heavy metal toxicity. Zhai et al[10] reported that LCB CCFM8610 exerts a protective effect against the acute toxicity of cadmium in mice. Such an effect is attributed to decreased cadmium intestinal absorption, tissue accumulation, and alleviation of hepatic and renal oxidative stress, and it results in amelioration of histopathological changes in the liver. The mechanism of mercuric chloride (MCH)-induced testicular toxicity is not fully understood. So, it is worthwhile to explore a novel mechanism involved in metal toxicities to allow for the emergence of a new treatment for its toxicity.

Until now, the exact molecular and genetic mechanisms involved in mercury toxicity are still not known. Thus, this study was conducted to discover a novel method for the treatment of MCH toxicity, which is widely found in many fields using the LCB and α-toco combination and to elucidate various molecular mechanisms underlying MCH-induced testicular atrophy. These mechanisms are involved in the formation of the main male sex hormone testosterone such as dehydroepiandrosterone (DHEA), mRNA of steroidogenic acute regulatory protein (STAR), 17β-hydroxysteroid dehydrogenase (17β-HSD), and 3β-hydroxysteroid dehydrogenase (3β-HSD). Also, for tracing protein expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and Bax as well as DNA fragmentation.

2 | MATERIALS AND METHODS

2.1 | Chemicals

MCH (Cat. 748-94-7; Sigma-Aldrich, St. Louis, MO), LCB (PM 310608; Axcan pharma S.A., France) and α-toco (T3251_SIGMA; Sigma-Aldrich). The primary antibodies for Bax and Nrf2 were obtained from Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA).

2.2 | Experimental animals

Thirty male 3-month-old Wistar albino rats weighing 250-280 g were obtained from the Experimental Animal Center, King Saud University. The animals were allowed to acclimatize in the laboratory for 1 week, at 22°C temperature. They were given a standard rat pellet diet and distilled water ad libitum. The use of animals was under the guidelines of King Saud University's ethics committee (KSU, SE, 19-38).

2.3 | Experimental design

Rats were allocated into five groups, each of six rats; groups were subjected to the treatment in this manner: group I was the control group, group II rats were injected (subcutaneous [SC]) with 5 mg/kg MCH daily for a continuous 5 days,[11] Group III (MCH + α-toco) rats received α-toco at a dose of 100 mg/kg/day,[12] Group IV (MCH + LCB) rats were treated with 6×10^{10} CFU of LCB 1.8701/kg in 1 mL of sterile normal saline[13] and group V (MCH + α-toco + LCB) rats were treated with α-toco and LCB at doses equal to the previous doses. All treatments were administered on the sixth-day post-MCH injection by oral route using oral-gavage for 3 weeks.

Rats were subjected to CO_2[14] and were killed by decapitation, blood samples were centrifuged at 3000 rpm. Then, the testes were collected. Parts of the testes were homogenized in phosphate buffer and centrifuged for 20 minutes at 1006.2 *g*. Other testes were kept frozen in liquid nitrogen for Western blot analysis. Finally, three testes were maintained in 10% formalin for histopathological examination.

2.4 | Biochemical analysis

Serum levels of tumor necrosis factor-α (TNF-α) (RTA00 USA R&D Systems, Inc), interleukin-6 (IL-6) (R6000B USA & Canada, R&D Systems, Inc), caspase-3 (ABIN 416112), testosterone, and DHEA (E-ELR0026) were determined using a rat enzyme-linked immunosorbent assay (ELISA) kit.

2.4.1 | Determination of reduced glutathione (GSH), superoxide dismutase (SOD), and lipid peroxide (malondialdehyde) in the testes

Reduced GSH was measured using the method of Ellman.[15] The SOD activity was determined using the method of Marklund and Marklund.[16] The lipid peroxidation level was measured employing the method of Uchiyama and Mihara.[17]

2.5 | Histological examination

Testes specimens were obtained and kept in 10% paraformaldehyde in phosphate buffer for 24 hours, and then embedded in paraffin wax at 57°C. Serial sections were made at widths of 4 μm using a Spencer 820 microtome. These sections were stained using hematoxylin and eosin and then were examined under a light microscope.[18]

2.6 | Determination of DNA fragmentation

Oligonucleosome-bound DNA was used to estimate DNA fragmentation using an ELISA kit (Boehringer, Mannheim, Germany).[19]

TABLE 1 Primer sequences used for RT-PCR

Primer	Primer sequences	Accession no.
β-actin	F: CCTGCTTGCTGATCCACA R: CTGACCGAGCGTGGCTAC	V01217
17β-HSD	F: GTGTGCACATTTTCCAAGGC R: TTTAACAAACTCATCGGCGG	NM 054007
3β-HSD	F: GCATTAACCCCACTCCCACT R: GGACCCTGACCTCCTTCAGA	NM 017265
STAR	F: CACAGTCATCACCCATGAGC R: AGCTCTGATGACACCGCTTT	NM031558.3

Abbreviations: 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; RT-PCR, reverse-transcription polymerase chain reaction; STAR, steroidogenic acute regulatory protein.

2.7 | Western blot analysis

Western blot analysis was employed to estimate the protein expression of Bax and Nrf2. Using the ECL-Plus detection system, the protein bands were visualized (Amersham Life Sciences, Little Chalfont, Buckinghamshire).[20]

2.8 | Gene expression using reverse-transcription polymerase chain reaction

mRNA of 17β-hydroxysteroid dehydrogenase (17β-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD), STAR were measured using a reverse-transcription polymerase chain reaction (RT-PCR) according to the method of Livak and Schmittgen.[21] Primers are allocated in Table 1.

2.9 | Statistical analysis

Data were expressed as means ± SEM for quantitative measures. Statistical difference between various groups was achieved using a one-way analysis of variance followed by the Tukey-Kramer post hoc test. The level of significance was set at $P < .05$, $P < .01$, and $P < .001$. Statistical tests were performed utilizing GraphPad Prism 5.00.

3 | RESULTS

Table 2 presents serum testosterone and DHEA in control as well as in all treated groups. It was revealed that MCH caused a significant decline in serum testosterone and DHEA compared to the control group ($P \leq .001$). Treatment with the antioxidants alone or together modulated the previously measured levels. The combination therapy achieved superlative results.

MCH elevated serum TNF-α, IL-6, and caspase-3 levels ($P \leq .001$) (Figure 1). Moreover, testicular GSH level and SOD activity were decreased, while the malondialdehyde (MDA) level was elevated in the MCH-administered group compared with the control group ($P \leq .001$) (Figure 1). However, treatment of the antioxidants in question alone or in combination decreased almost all of the above distorted biochemical parameters compared with the MCH-administered group.

mRNA expression of STAR, 17β-HSD, 3β-HSD was downregulated in testicular tissue upon MCH administration ($P \leq .001$) (Figure 2).

Protein expression of Bax was upregulated while that of Nrf2 was downregulated upon MCH administration compared to the control group ($P \leq .001$) (Figure 3). In contrast, MCH enhanced DNA fragmentation in the testicular tissues upon MCH administration (Figure 4).

MCH caused the formation of a fibrotic histopathological structure of mature active seminiferous tubules. Conversely, treatment with the antioxidants individually or in combination modulated these expressions compared with the MCH-administered group and restored the regular histopathological architecture of mature active seminiferous tubules with a complete spermatogenic series. Single or combined treatment also decreased tissue destruction and restored the spermatogonia layer. Treatment with the antioxidants alone or together modulated most of the previously measured parameters; however, the combination regimen exhibited the most notable enhancement results (Figure 5).

4 | DISCUSSION

Mercury is used in industrial and pharmacological sectors.[22] GSH depletion by mercury may trigger ROS production that enhances lipid, protein, and DNA oxidation,[23] this can severely affect spermatogonia.[24] Spermatozoal membranes are rich in polyunsaturated fatty acids, hence, they are susceptible to ROS attack, and hence, loss of the integrity of the

TABLE 2 Serum testosterone and DHEA levels in control and MCH-injected group as well as in all treated groups

	Parameters				
Groups	Con	MCH	MCH + α-toco	MCH + LCB	MCH + α-toco + LCB
Testosterone (ng/mL)	4.29 ± 0.09	2.36 ± 0.19***	3.2 ± 0.13ϯϯ	3.35 ± 0.16ϯϯϯ	3.55 ± 0.33ϯϯϯ
DHEA (ng/mL)	0.278 ± 0.01	0.143 ± 0.01***	0.213 ± 0.01ϯϯϯ	0.205 ± 0.01ϯϯϯ	0.255 ± 0.01ϯϯϯ

Note: Data are mean ± SEM (n = 6).

Abbreviations: DHEA, dehydroepiandrosterone; LCB, *Lactobacillus plantarum*; MCH, mercuric chloride; α-toco, α-tocopherol.

***$P < .001$ vs control

ϯϯ$P < .01$ vs MCH group.

ϯϯϯ$P < .001$ vs MCH-injected group.

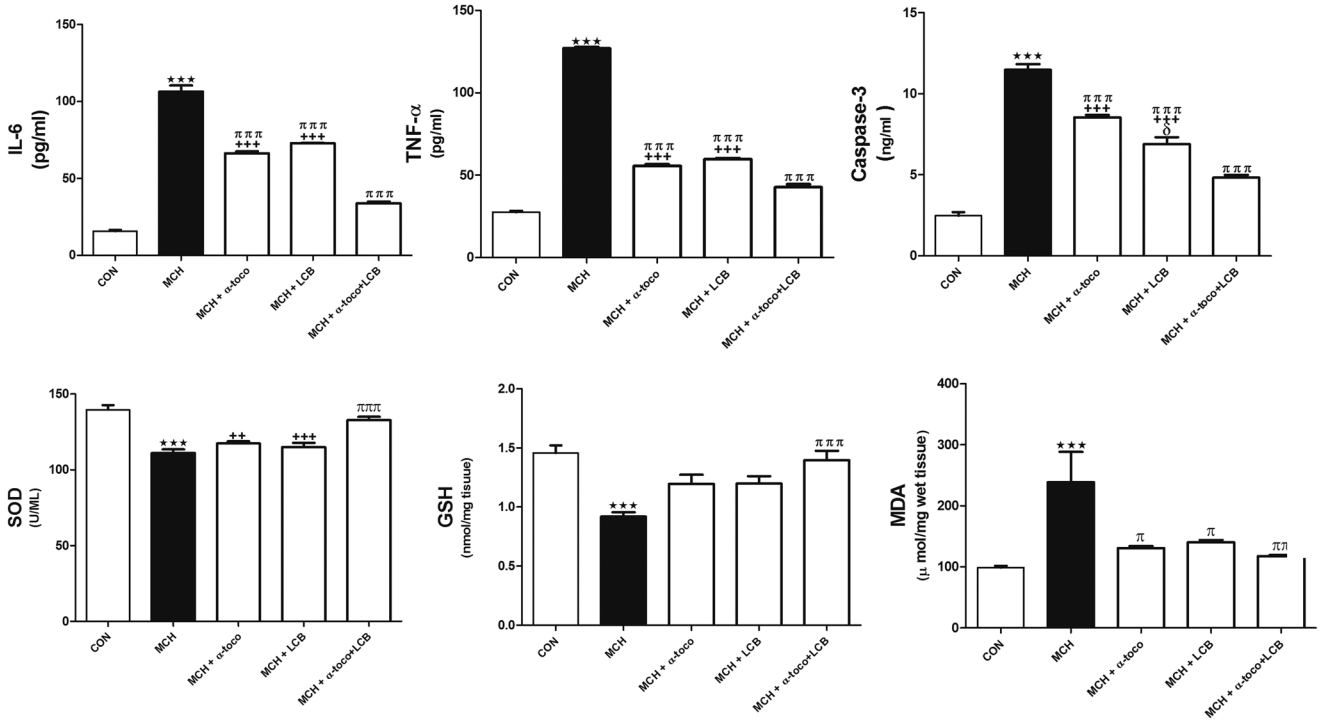

FIGURE 1 α-Toco and LCB alone or together reduced serum levels of IL-6, TNF-α, caspase-3, and testicular MDA, whereas GSH and SOD activity were elevated in MCH-administered rats. GSH, glutathione; IL-6, interleukin-6; LCB, *Lactobacillus plantarum*; MCH, mercuric chloride; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; α-toco, alpha-tocopherol. Data are Mean ± SEM (n=6). $^{***}P < .001$ vs control, $^{\pi\pi\pi}P < .001$ vs MCH-injected group, $^{\delta}P < .05$ vs α-toco treated group, and $^{+++}P < .001$ vs the combination treated group

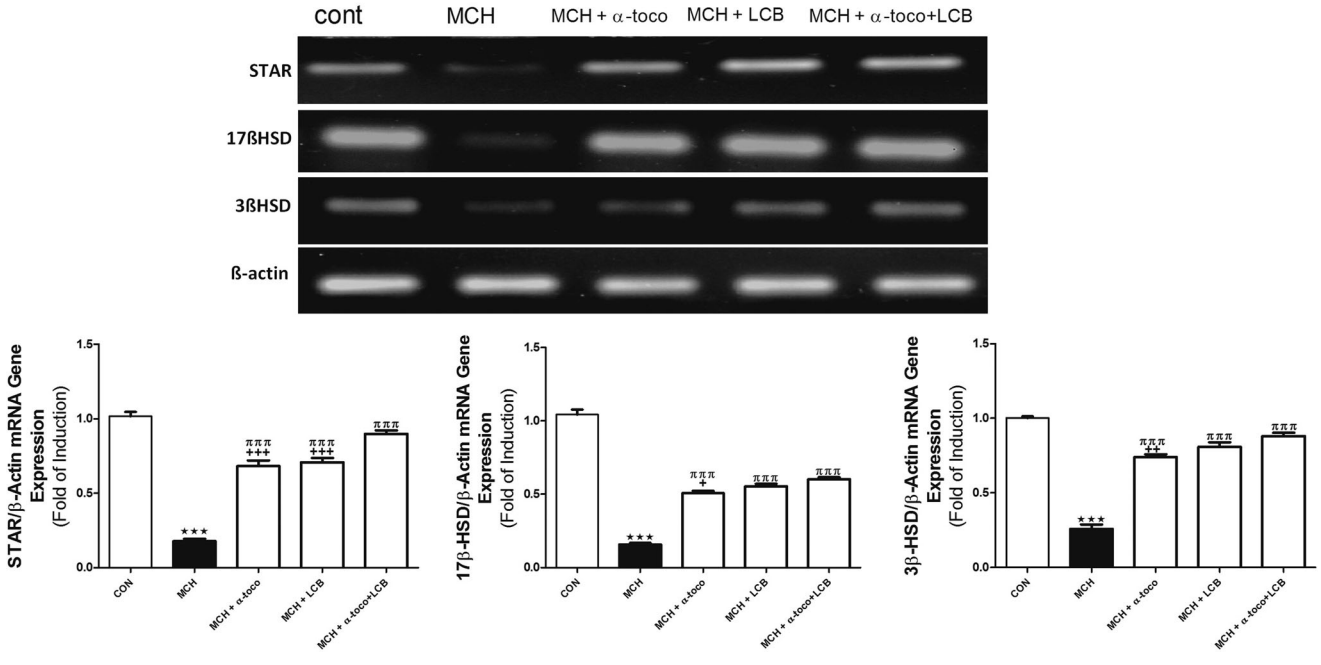

FIGURE 2 α-toco and LCB and their combination increased messenger RNA gene expression of STAR, 17βHSD, and 3βHSD in MCH-injected rats. Data are mean ± SEM (n = 6). $^{***}P < .001$ vs control, $^{\pi\pi\pi}P < .001$ vs MCH-injected group, and $^{+++}P < .001$ vs the combination-treated group. 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; LCB, *Lactobacillus plantarum*; MCH, mercuric chloride; SEM, standard error of the mean; STAR, steroidogenic acute regulatory protein; α-toco, alpha-tocopherol

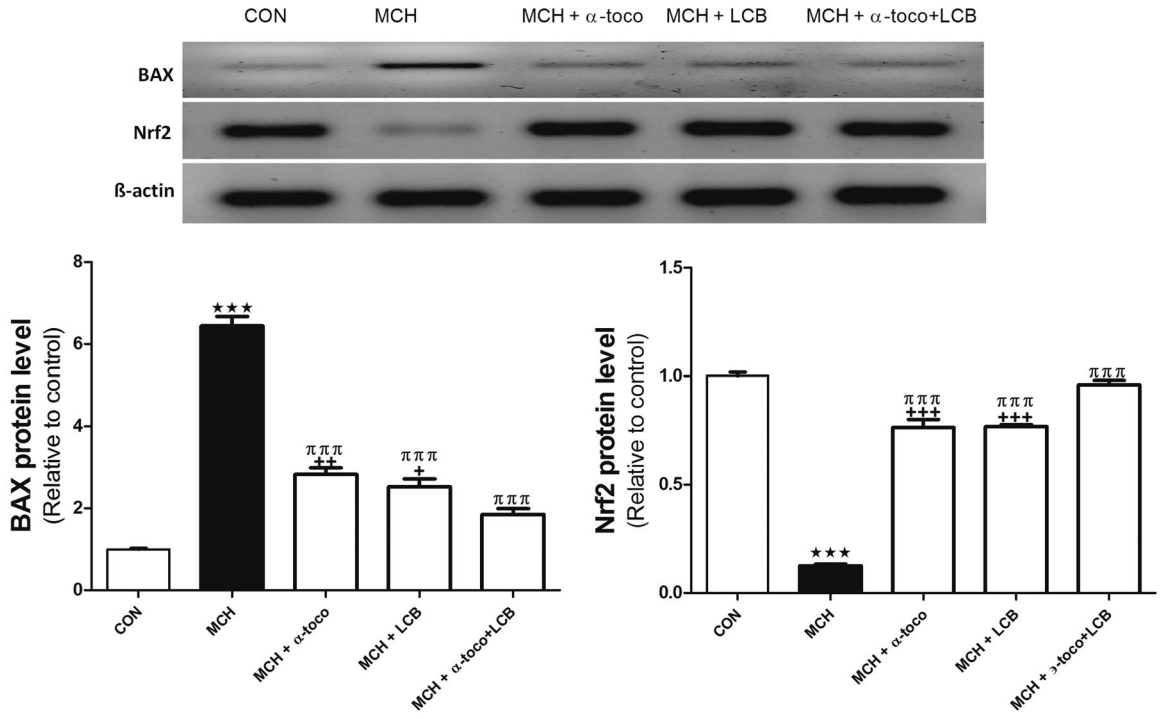

FIGURE 3 α-toco and LCB alone or together downregulated protein expression of BAX and increased Nrf2 protein expression in MCH-injected rats. Data are mean ± SEM (n = 6). $^{***}P < .001$ vs control, $^{\pi\pi\pi}P < .001$ vs MCH-injected group, and $^{+++}P < .001$ vs the combination-treated group. LCB, *Lactobacillus plantarum*; MCH, mercuric chloride; SEM, standard error of the mean; α-toco, alpha-tocopherol

mitochondrial membrane and enhancing lipid peroxidation leads to a defect in sperm morphology and subsequent decrease in sperm motility upon mercury exposure.[25,26]

In the present study, MCH injection elevated the levels of serum TNF-α, IL-6, while GSH level and SOD activity were decreased and MDA was increased in testicular tissue. Cotreatment with α-toco and LCB either alone or together ameliorates these levels. It was found that supplementation of α-toco decreased oxidative stress induced by MCH[27] and also plays a critical role in reducing mercury absorption from the gastrointestinal tract.[28] However, LCB can bind to heavy metals in vitro[6] and displays antioxidative properties and anti-inflammatory activity in human subjects.[7,8,29] LCB administration provided significant

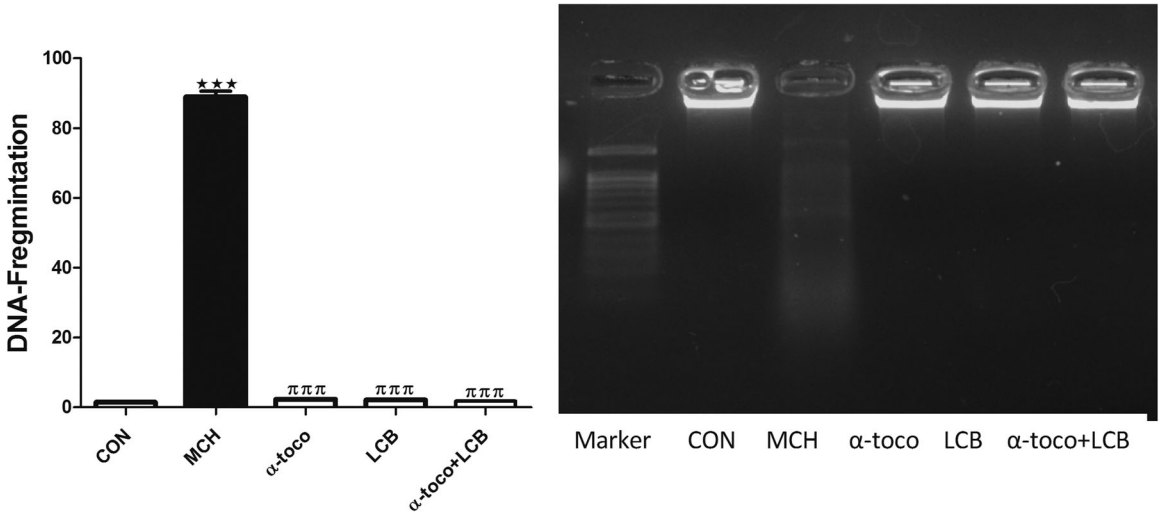

FIGURE 4 α-toco and LCB alone or together downregulated DNA fragmentation in MCH-injected rats. Data are mean ± SEM (n = 6). $^{***}P < .001$ vs control and $^{\pi\pi\pi}P < .001$ vs MCH group. LCB, *Lactobacillus plantarum*; MCH, mercuric chloride; SEM, standard error of the mean; α-toco, alpha-tocopherol

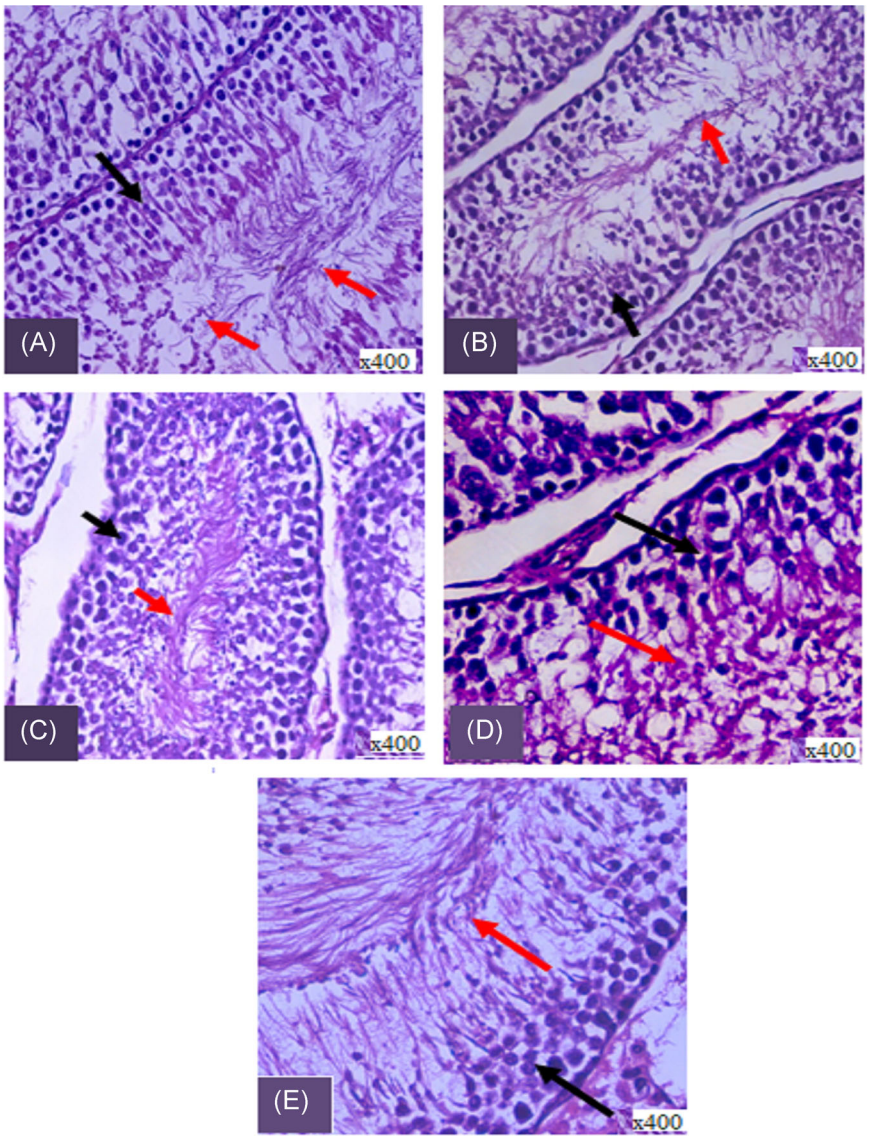

FIGURE 5 A, Section from testes of the normal control group showing the normal histopathological structure of mature active seminiferous tubules with complete spermatogenic series (black arrows), with a large number of spermatocytes in the center (red arrow). B, The section from testes of mercuric chloride group showing the fibrotic histopathological structure of mature active seminiferous tubules (black arrow) with a decreased number of spermatocytes in the center (red arrow). C, The section from testes of the α-tocopherol (α-toco) group showing the normal histopathological structure of mature active seminiferous tubules with a complete spermatogenic series (black arrows), and with a large a number of spermatocytes in the center (red arrow). D, The section from testes of *Lactobacillus plantarum* (LCB) group showing the normal histopathological structure of mature active seminiferous tubules with a complete spermatogenic series (black arrows), and with few spermatocytes in the center (red arrow). E, The section from testes of α-toco and LCB group shows the normal histopathological structure of mature active seminiferous tubules with a complete spermatogenic series (black arrows), and with few spermatocytes in the center (red arrow)

protection against mercury toxicity, hence it decreases the mercury level in the hepatic and renal tissues and prevents modifications in the levels of GSH peroxidase and SOD.[29]

Mercury exhibits a significant decrease in the levels of testosterone, FSH, LH, and prolactin suggesting the dysfunction of the pituitary-testicular axis and harmfully affects reproductive systems.[30] DHEA is a steroid hormone, rapidly transformed into androstenedione by 3β-HSD in peripheral target tissues, then it undergoes additional conversion to testosterone a by 17β-HSD and aromatase, respectively.[31,32]

The present study revealed that MCH caused a significant decline in serum testosterone and DHEA levels compared with the control group ($P \leq .001$). Treatment with the antioxidants alone or together modulated the previous biochemical parameters. It was reported that MCH significantly decreased the activity of 3β-HSD and 17β-HSD, causing a reduction in plasma testosterone levels, and testicular sperm count as well as loss in sperm motility and an increase in the levels of oxidative stress in the testes of mice. 7β-HSDs play a critical role in the conversion of 17-ketosteroids to 17-hydroxysteroids. STAR is a

transport protein that regulates cholesterol transfer within the mitochondria, this is the rate-limiting step in the production of steroid hormones.[33,34] Testicular injuries are established by an increase in sperm abnormalities and a decrease in testosterone levels.[9]

Herein, MCH downregulated mRNA expression of 3β-HSD, 17β-HSD, and STAR. Histopathological examination revealed that MCH injection resulted in a fibrotic structure in mature active seminiferous tubules. However, treatment with antioxidants in question restored these altered genes and the restored normal architecture of the testes.

The release of cytochrome *c*, the major step in the mitochondrial pathway of apoptosis, is controlled by Bax, Bcl-2, and excessive DNA fragmentation.[35] Nrf2, a factor sensing the presence of oxidative stress, regulates transcription of genes encoding for cytoprotective enzymes crucial for maintaining cellular homeostasis.[36] It was reported that MCH decreased Nrf2 level, increased Bax and decreased Bcl-2.[37]

In the present study, the serum level of caspase-3 and Bax protein expression was elevated while that of Nrf2 was downregulated, DNA fragmentation was dramatically increased in the testes of rats injected with MCH. However, treatment with LCB and α-toco decreased the level of caspase- 3, testicular Bax, and the DNA fragmentation, while Nrf2 protein expression was elevated.

5 | CONCLUSION

The LCB and α-toco combination is considered a promising candidate for the treatment of testicular dysfunction induced by MCH. The expression of STAR, 17β-HSD, 3β-HSD, Bax, and Nrf2 may present a molecular target that is involved in MCH toxicity and treatment.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this study through (RG-1440-017).

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REFERENCES

[1] D. A. Geier, P. G. King, L. K. Sykes, M. R. Geier, *Indian J. Med. Res.* **2008**, *128*, 383.
[2] M. Sevcikova, H. Modra, A. Slaninova, Z. Svobodova, *Vet. Med.* **2011**, *56*, 537.
[3] S. J. Flora, V. Pachauri, *Int. J. Environ. Res. Public Health* **2010**, *7*, 2745.
[4] I. D. Coulter, M. L. Hardy, S. C. Morton, L. G. Hilton, W. Tu, D. Valentin, P. G. Shekelle, *J. Gen. Intern. Med.* **2006**, *21*, 735.
[5] R. Agarwal, S. K. Goel, R. Chandra, J. R. Behari, *Environ. Toxicol. Pharmacol.* **2010**, *29*, 70.
[6] T. Halttunen, M. Collado, H. El-Nezami, J. Meriluoto, S. Salminen, *Lett. Appl. Microbiol.* **2008**, *46*, 160.
[7] T. Kullisaar, E. Songisepp, M. Mikelsaar, K. Zilmer, T. Vihalemm, M. Zilmer, *Brit. J. Nutr.* **2003**, *90*, 449.
[8] H. S. Ejtahed, J. Mohtadi-Nia, A. Homayouni-Rad, M. Niafar, M. Asghari-Jafarabadi, V. Mofid, *Nutrition* **2012**, *28*, 539.
[9] G. E. El-Desoky, S. A. Bashandy, I. M. Alhazza, Z. A. Al-Othman, M. A. Aboul-Soud, K. Yusuf, *PLOS ONE* **2013**, *8*, e59177.
[10] Q. Zhai, G. Wang, J. Zhao, X. Liu, F. Tian, H. Zhang, W. Chen, *Appl. Environ. Microbiol.* **2013**, *79*, 1508. https://doi.org/10.1128/AEM.03417-12
[11] N. C. Peixoto, T. Roza, E. M. Flores, M. E. Pereira, *Toxicol. Lett.* **2003**, *146*, 17.
[12] J. M. Yousef, G. Chen, P. A. Hill, R. L. Nation, J. Li, *J. Antimicrob. Chemother.* **2012**, *67*, 452.
[13] B. Li, D. Jin, S. Etareri Evivie, N. Li, F. Yan, L. Zhao, F. Liu, G. Huo, *Toxins* **2017**, *9*, 301. https://doi.org/10.3390/toxins9100301
[14] G. P. Boivin, D. L. Hickman, M. A. Creamer-Hente, K. R. Pritchett-Corning, N. A. Bratcher, *J. Am. Assoc. Lab. Anim. Sci.* **2017**, *56*, 491.
[15] G. L. Ellman, *Arch. Biochem. Biophys.* **1959**, *82*, 70.
[16] S. Marklund, G. Marklund, *Eur. J. Biochem.* **1974**, *47*, 469.
[17] M. Uchiyama, M. Mihara, *Anal. Biochem.* **1978**, *86*, 271.
[18] A. Smith, J. Bruton, *Eur. J. Clin.* **1978**, *31*, 298.
[19] M. Leist, F. Gantner, I. Bohlinger, P. G. German, G. Tiegs, A. Wendel, *J. Immunol.* **1994**, *153*, 1778.
[20] L. Jackson, K. Wu, Y. Mahida, D. Jenkins, C. Hawkey, *Gut* **2000**, *47*, 762.
[21] K. J. Livak, T. D. Schmittgen, *Methods* **2001**, *25*, 402.
[22] R. A. Sharma, W. P. Steward, A. J. Gescher, *Adv. Exp. Med. Biol.* **2007**, *595*, 453. https://doi.org/10.1007/978-0-387-46401-5_20
[23] P. Jezek, L. Hlavata, *Int. J. Biochem. Cell Biol.* **2005**, *37*, 2478.
[24] M. A. Boujbiha, K. Hamden, F. Guermazi, A. Bouslama, A. Omezzine, A. Kammoun, A. E. Feki, *Reprod. Toxicol.* **2009**, *28*, 81.
[25] V. Mandava, B. G. Rao, *Toxicol. In Vitro* **2008**, *22*, 935.
[26] E. Celikoglu, A. Aslanturk, Y. Kalender, *Braz. Arch. Biol. Technol.* **2015**, *58*, 4.
[27] L. Su, M. Wang, S. T. Yin, H. L. Wang, L. Chen, L. G. Sun, D. Y. Ruan, *Ecotoxicol. Environ. Safety* **2008**, *70*, 483.
[28] A. Jančová, P. Massányi, P. Nad', B. Koréneková, M. Skalická, J. Drábeková, I. Baláž, *Ekológia* **2006**, *25*, 19.
[29] V. Ramalingam, V. Vimaladevi, S. Rajeswary, V. Suryavathi, *J. Environ. Biol.* **2003**, *24*, 401.
[30] H. Moussa, L. Hachfi, M. Trimèche, M. F. Najjar, R. Sakly, *Andrologia* **2011**, *43*, 23.
[31] M. S. Barkeley, B. D. Goldmass, *Horm. Behav.* **1977**, *8*, 208.
[32] A. E. A. Moneim, *Neural Regener. Res.* **2015**, *10*, 881. https://doi.org/10.4103/1673-5374.158336
[33] V. Luu-The, *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 143.
[34] P. R. Manna, M. T. Dyson, D. M. Stocco, *Mol. Hum. Reprod.* **2009**, *15*, 321. https://doi.org/10.1093/molehr/gap025
[35] C. S. Martinez, A. G. Escobar, J. G. Torres, D. S. Brum, F. W. Santos, M. J. Alonso, M. Salaices, D. V. Vassallo, F. M. Peçanha, F. G. Leivas, G. A. Wiggers, *J. Toxicol. Environ. Health A* **2014**, *77*, 143. https://doi.org/10.1080/15287394.2014.867202
[36] A. Wang, S. Wang, Y. Jiang, M. Chen, Y. Wang, L. Lin, *Food Funct.* **2016**, *7*, 2074.
[37] H. Zhang, X. Tan, D. Yang, J. Lu, B. Liu, R. Baiyun, Z. Zhang, *Oncotarget* **2017**, *8*, 40982. https://doi.org/10.18632/oncotarget.17334

How to cite this article: Fadda LM, Alhusaini AM, Al-Qahtani QH, Ali HM, Hasan IH. Role of α-tocopherol and *Lactobacillus plantarum* in the alleviation of mercuric chloride-induced testicular atrophy in rat's model: Implication of molecular mechanisms. *J Biochem Mol Toxicol*. 2020;34:e22481. https://doi.org/10.1002/jbt.22481