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RESEARCH ARTICLE

Effect of *Lactobacillus brevis* (MG000874) on antioxidant-related gene expression of the liver and kidney in D-galactose-induced oxidative stress mice model

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Abstract

Oxidative stress (OS) is a phenomenon induced by excessive production and accumulation of reactive oxygen species (ROS) in living cells. These increased ROS productions connected, coupled with many neurological and physiological diseases. Several antioxidants were utilized recently to combat OS, and lactic acid bacteria have a potent radical-scavenging activity to minimize OS. The present work was designed to find out the protective effects of *Lactobacillus brevis* MG000874 (*L. brevis* MG000874) against oxidative injuries induced by D-galactose (D-gal) in vivo and to explore the gene expression of OS-related gene mice. Sixty male mice were randomly split into six groups. The first four groups were different control groups as no treatment (N), positive (G), probiotic (B), and ascorbic acid (A); the remaining two groups were treatment groups such as probiotic treatment (BG) and ascorbic acid treatment (AG). *L. brevis* MG000874 (0.2 ml of 10^{10} CFU/ml) and ascorbic acid (0.2 ml of 25 mg/ml) were administered orally daily for 5 weeks. It was revealed that these significantly affect the weight of treated mice: 40.22 ± 1.5 and 33.0 ± 0.57 g on days 0 and 36, respectively. D-gal induction in mice declined the levels of SOD and CAT determined by spectrophotometer. Administration of *L. brevis* MG000874 improved the antioxidant status of the stress mice and recovered the antioxidant activities of SOD and CAT enzymes. In addition, *L. brevis* MG000874–altered gene expression of OS marker at the messenger RNA (mRNA) levels was determined by RT-PCR in the mouse model. *L. brevis* MG000874 significantly improved the GST, GPX, SOD, CAT, and ß-actin levels in the kidney and the liver of the D-gal-induced mice ($p < 0.05$). Moreover, the histological investigation indicated that *L. brevis* MG000874 mitigated damage to the kidney and liver effectively in mice induced by D-gal. Therefore, it could be concluded from the current results that *L. brevis* MG000874 may act as a powerful antioxidant agent, and this study can provide the baseline data for drug development against OS-linked diseases.

Keywords Aging · Antioxidant · D-galactose · Lactic acid bacteria · Mice · Oxidative stress · RT-PCR

Introduction

Oxidative stress (OS) is an imbalance between the production and the accumulation of free radical oxygen species (FROS). These FROS thus adversely alter the properties of lipids, proteins, and DNA, which in turn triggered many diseases (Ghazi et al. 2022). FROS is one of the most common action mechanisms in aging as well (Stroustrup 2018; Rehman et al. 2022). The stability of FROS and antioxidants becomes unstable in the case of the OS. The living systems always seek to maintain the level of FROS for their normal functions. Although, elevated levels of FROS directly affected the activity of the antioxidant enzyme defense system (AEDS) and non-enzymatic proteins (GSH), which influenced the homeostasis of the

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whole biological defense system (Liguori et al. 2018; Arya et al. 2021).

It has been suggested that the OS might be regulated by the configuration of gut microbiota (Wells et al. 2020). The sources of these gut microbiota are different via food and probiotic supplements. Probiotics may activate, modify, and adjust the host's immune status by prompting the stimulation of particular genes of confined host cells (El-Sayed et al. 2021). Probiotics comprising Lactobacillus and Bifidobacterium have appealed considerable attention due to their vital role in health promotion. The important purpose of isolating probiotics from all over the world is to scale up the beneficial global food system by the production of healthy organisms, particularly with prolonged existence (Fanzo et al. 2020). The antioxidant capability of lactic acid bacteria (LAB) and their constituents (e.g., Proteins and exopolysaccharides) are normally specified and considered as standard for the assessment of probiotic functions (Li et al. 2015). Assessment of antioxidant properties of LAB that comprises in vitro oxidation reaction system (free radical scavenging, metal ion chelation, and lipid peroxidation inhibition) and in vivo animal confirmation of the antioxidant role of natural additives may be supported by Yadav et al. (2019), Unban et al. (2021) Asan et al. (2022), and Li et al. (2022). Hence, the LAB having the quenching ability of FROS becomes more significant in investigating the mechanism of antioxidants. The literature revealed that a high concentration of D-galactose (D-gal) helped in the generation of FROS by the accumulation of advanced glycation end products, as a result of the OS and cellular damage produced in the tissues (Shwe et al. 2018). In the current era, several reports about the antioxidant evaluation of LAB have been published. However, a systematic evaluation of the antioxidant mechanism from the gene expression level of mice has been reported rarely. In our previous study, *L. brevis* MG000874 was extracted from the camel and used to investigate its antioxidant ability against D-gal (Noureen et al. 2019). The current study was therefore to explore the antioxidant ability of *L. brevis* MG000874 by investigating the gene expression of OS-related gene markers in mice.

Materials and methods

Hydrogen peroxide (H_2O_2) (Daejung, Siheung-si, Korea), MRS broth (Oxide, UK), sodium chloride (Merck, Germany), phosphate-buffered saline, tablet (sigma), (pyrogallol (sigma), D-gal, (Germany), ascorbic acid (Germany), RNA extraction kit, Syber green master mix, and cDNA extraction kit were procured from a local dealer.

Bacterial culture

The bacterial strain (*L. brevis* MG000874) was isolated from the camel intestinal tract as the method used in our previous article by Noureen et al. (2019). The isolated strains of *L. brevis* MG000874 were incubated at 37 °C by culturing in MRS broth (pH 6.6 ± 02) overnight. For further use in treatments, these isolates were separated by centrifugation at 3000 rpm and suspended in a PBS buffer.

In vivo evaluation of antioxidant prospective

Sixty days old male albino mice (*Mus musculus*, weight: 40.0 ± 2.0 g, $n = 60$) were set aside in cages under schematized circumstances (temperature 22 ± 2 °C, dampness 45 ± 5%, 12-h light/dark cycles). During the experiments, open access was provided for water and food. The mice were indiscriminately divided into six groups after 1 week of acclimatization. These groups were negative control group (N)—no treatment, probiotic cells, and treatment group with *L. brevis* MG000874 (B)—and positive control group (G)D-gal treatment group (300 mg/BW), ascorbic acid treatment group (A), D-gal and *L. brevis* MG000874 treatment group (BG), and D-gal and ascorbic acid treatment group (AG).

Dose setting

L. brevis MG000874 (MG000874) and ascorbic acid were given via gastric gavage. D-gal was injected at the dose of 300 mg/kg BW/day through a subcutaneous route for 5 weeks, on behalf of many pilot studies for a quick aging process (data not included). The animals were anesthetized after 5 weeks of treatment. Organ index, kidney, and liver tissue homogenization were prepared according to the Noureen et al. (2019) method.

Estimation of antioxidants

The antioxidant level in the liver and kidney was estimated through SOD and CAT. Briefly, in the SOD assay, the reaction mixture was incubated at 25 °C for 20 min comprising 2.8 ml Tris HCl buffer (0.01 mmol/l, pH 85) and 0.1 ml sample. Absorbance was noted at 412 nm for 3 min by the addition of 0.1 ml pyrogallol of 8 mmol/l in the mixture.

Furthermore, in catalase, the addition of 0.1 ml of H_2O_2 (0.1 mmol/l) in the reaction mixture initiated the reaction which consisted of 190 ml PBS of 1.0 mmol/l (pH 6.8) and 0.1 ml sample. At 240 nm, the variation in absorbance was

noted for 3 min. Catalase and SOD activities in tissue were calculated according to Noureen et al. (2019).

Histopathological studies

For the histopathological study, tissues of liver and kidney samples (nearly 1 cm^2 thick) were fixed in a 10% formalin solution and transferred to different grades of absolute alcohol dehydration. Then, the samples were fixed in paraffin and 3–4-µm-thick slices. These slices were transferred to glass slides and rehydrated and then stained with hematoxylin and eosin staining. Prepared slides were observed under the light microscope having a fitted camera (Labomed, USA) for histopathological examination (Qiu et al. 2017; Iqbal et al. 2022).

Extraction of RNA and preparation of cDNA

RNA was extricated from tissues following the TRIzol method (Brown et al. 2018). The purity and quantity of attaining RNA (ribonucleic acid) were estimated through ND-1000 (Nanodrop, Thermo Fisher Scientific). cDNA (complementary DNA) was made through Synthesis Kit (First-Strand cDNA kit).

RT-PCR

RNA expression of SOD, CAT, GST, GPX, and ß-actin was investigated using RT-PCR. Each primer sequence's detail is provided in Table 1. Concisely, the qPCR reaction mixture (20 µl) contained CDNA (0.1 µl), primer (1.4 µl), SYBR Green master mix (12.5 µl), and nuclease-free water (9.5 µl). Forty cycles of qPCR were done on an RT-PCR system (Bio-Rad CFX): 3 min denaturation at 95 °C, 10 s annealing at 54–59 °C, and 30 s elongation at 72 °C. The expression of mRNA was determined by average quantification cycle (Cq) values. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene (Eissa et al. 2016). The expression was calculated by the following:

$$\text{Normalized expression ratio(NER)} = 2 - \Delta\Delta\text{Ct}$$

$$\Delta\Delta\ \text{Ct} = \Delta\ \text{Ct(SOD)} - \Delta\ \text{Ct(GAPDH)}$$

$$\Delta\ \text{Ct(SOD)} = \text{Sample Ct(SOD)} - \text{reference sample Ct(SOD)}$$

$$\Delta\ \text{Ct(GAPDH)} = \text{Control Ct(GAPDH)} - \text{reference of control Ct(GAPDH)}$$

Statistical analysis

One-way ANOVA was performed for group comparison (*p-values* ˂ 0.05, significant: Tukey's test) by using IBM SPSS 21.0 (Inc., Chicago, IL). Tests were done in triplicate, and their outcomes were displayed as the standard error of means (SEM) (Lin et al. 2018).

Results

Organ index

Table 2 reveals that the indexes of the liver and kidney in the stress group (G) were significantly lesser than those in the normal group (N: $p < 0.05$), presenting that induction of the D-gal was the cause of organ stress or aging. However, the liver and kidney index of treated groups BG and

Table 1 Primer sequences for qRT-PCR

Gene	Primer sets	Annealing temperature (°C)	References
SOD	(F) 5′ AACCAGTTGTGTTGTCAGGAC3′ (R) 5′ CCACCATGTTTCTTAGAGTGAGG 3′	55	Li et al. (2020)
CAT	(F) 5′ ACGAGATGGCACACTTTGACAG 3′ (R) 5′ TGGGTTTCTCTTCTGGCTATGG 3′	55	Li et al. (2020)
GST	(F) 5′GCTGGAGTGGAGTTTGAAGAA3′ (R) 5′ GTCCTGACCACGTCAACATAG3′	59	Shen et al. (2015)
GPX	(F) 5′ AGTCCA CCGTGTATGCCTT CT 3′ (R) 5′ GAGACGCGA CATTCTCAATGA 3′	55	Zheng et al. (2016)
ß-actin	(F) 5′ TCACTATCGGCAATGTGCGG 3′ (R) 5′ GCTCAGGAGGAGCAATGATG 3′	55	Almaghrabi (2015)
GAPDH	(F) 5′ TGCAGTGGCAAAGTGGAGAT 3′ (R) 5′ TTTGCCGTGAGTGGAGTCAT 3′	54	Li et al. (2020)

F forward, *R* reverse

Table 2 Effect of treatments on body weight and kidney and liver/body weight ratio

Parameters	Treatment groups					
	N	B	A	G	BG	AG
Body weight (g)	40.00 ± 1.15^{a}	41.66 ± 2.23^{a}	41.02 ± 0.88^{a}	33.0 ± 0.57^{b}	37.66 ± 0.66^{ab}	37.01 ± 0.88^{ab}
Kidney/bodyweight ratio (g)	0.24 ± 0.01^{ab}	0.28 ± 0.01^{a}	0.27 ± 0.01^{ab}	0.20 ± 0.01^{c}	0.24 ± 0.01^{ab}	0.23 ± 0.01^{bc}
Liver/body weight ratio (g)	5.95 ± 0.76^{b}	6.33 ± 0.08^{a}	6.23 ± 0.08^{a}	4.9 ± 0.06^{c}	5.55 ± 0.12^{b}	5.53 ± 0.12^{b}

Data represent mean ± SEM; means with different superscript (a, b, c) in same tier significantly vary ($P < 0.05$)

N normal group, *G* D-gal treatment group, *A* ascorbic acid treatment group, *B L. brevis* MG000874 treatment group, *BG* galactose and *L. brevis* MG000874 treatment group, *AG* D-gal and ascorbic acid treatment group

AG was significantly higher than those in the G group and showed more observable effects on inhibiting organ aging.

Enzyme activities of SOD and CAT in liver and kidney tissues

The antioxidant activity of the enzyme can exactly reveal oxidative injury. So, we used the two indicators of OS SOD and CAT. The enzyme activities of SOD and CAT in the liver and kidney of the G group were noticeably the lowest (Fig. 1). But the levels of these enzymes were significantly improved in the treatment of BG or AG. Particularly, the levels of the SOD and CAT enzymes in the B or A group were very close to the N group, illustrating that *L. brevis* MG000784 and ascorbic acid were better at delaying the stress factor.

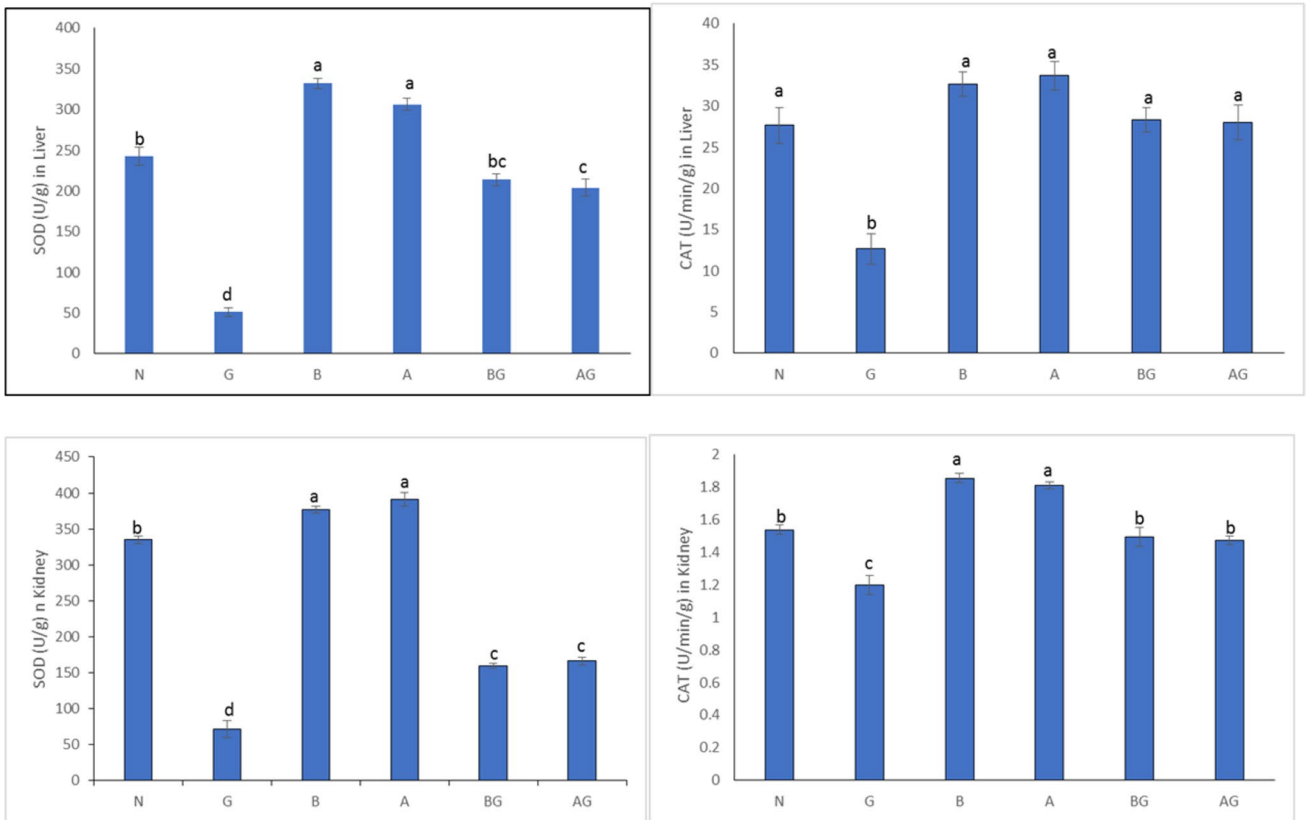

Fig. 1 Effects of *L. brevis* MG000874and ascorbic acid on D-Gal induced changes in SOD and CAT of liver and kidney.N: control; G: d-gal; B: *L. brevis*MG000874; A: Ascorbic Acid; BG: group getting both D-Gal and *L. brevis* MG000874; AG: Group gettingAscorbic acid and D-Gal. Data represent Mean ± SEM; means with different-superscript letter (a, b, c, d) exhibits significant differences ($P < 0.05$)

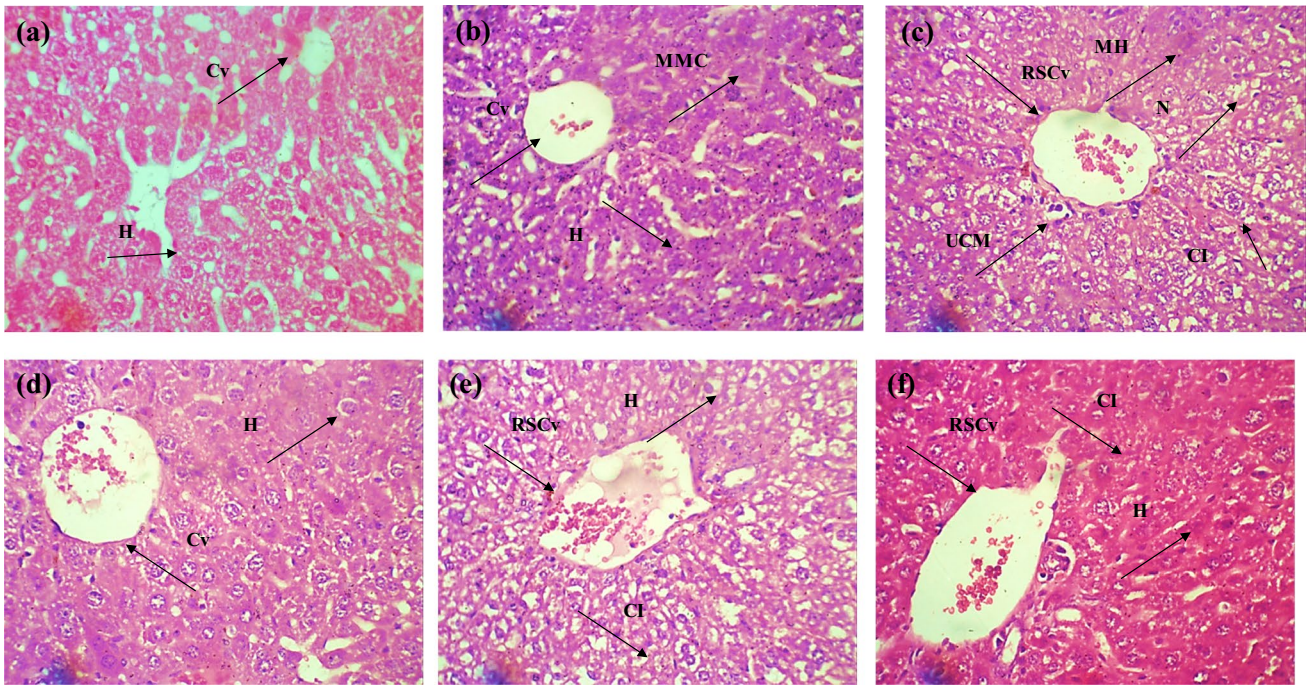

Fig. 2 Illustration of histological alterations in kidney tissues (H&E;X400) (**a**) normal kidney tissues, (**b**) *L.brevis* MG000874 group, (**c**) B: G: D-gal group, (**d**) A: Ascorbic Acid group,(**e**) BG: D-Gal + *L. brevis* MG000874,(**f**) AG: Ascorbic acid + D-Gal., showed hepatocytes (H), central vein (Cv),messy hepatocytes (MH), cell inflammation (CI), uneven cell margin (UCM), roughshape of central vein (USCv) and mediummessy cells (MMC)

Histological examination of mouse liver and kidney

The histological alteration in the liver of treated mice is given in Fig. 2. It was evident from the results that the morphology of the hepatocytes in the N group was consistent with no change (Fig. 2a). However, notable alterations were recorded in the positive control (G) including messy hepatocytes (MH), cell inflammation (CI), necrosis (N), and rough shape of the central vein (RSCv) (Fig. 2c). The groups B and A (Fig. 2b and d) exhibit the normal histology as in group N except for some degree of inflammation in hepatocytes. The treatment groups BG and AG (Fig. 2 f and g), therefore, showed the protective effects of *L. brevis* MG000874 and ascorbic acid, respectively, against D-gal-induced toxicity or OS. The kidney photomicrograph were shown (Fig 3) that the Bowman's capsule of N and B group had normal distal convoluted tubules, vascular pole, renal parenchyma, inner cellular layer of podocytes and outer squamous capsular cells.

Liver gene expression content

The overall changes in the mRNA expressions of antioxidant enzymes of all groups in liver tissue are summarized in Fig. 4. These gene expressions consisted of SOD, CAT, GST, GPX, and B-actin, and they were determined by RT-PCR. The expression levels of SOD, CAT, GST, GPX, and B-actin in group N were the highest. However, in the D-gal group (G), the mRNA expression levels of the above genes were downregulated. Raised SOD and CAT mRNA levels were also noted in mice treated with *L. brevis* MG000874 (B) or ascorbic acid (A) as compared with N. These results were in agreement with the corresponding enzyme activity results (Fig. 1). In treatment groups, BG and AG, the expressions of SOD, CAT, GST, GPX, and B-actin were significantly improved ($p < 0.05$).

Kidney gene expression content

The gene expression level of Kidney tissue is presented in Fig. 5. The mRNA expression of SOD, CAT, GST, GPX, and B-actin of the normal group (N) in comparison to the D-gal group (G) was the lowest, and these results were in agreement with Fig. 1. In the treatment groups, SOD, CAT, GPX, GST, and B-actin mRNA expressions and B-actin were increased. The expression of *L. brevis* MG000874 alone in the treatment group (B) was highest in comparison to the N group, which was stronger than that of ascorbic acid.

Discussion

LABs are abundant and ubiquitous, found in nutrient-rich habitats, including animal feed, humans, plants, and soil. They possess specific enzymes and produce many chemical molecules

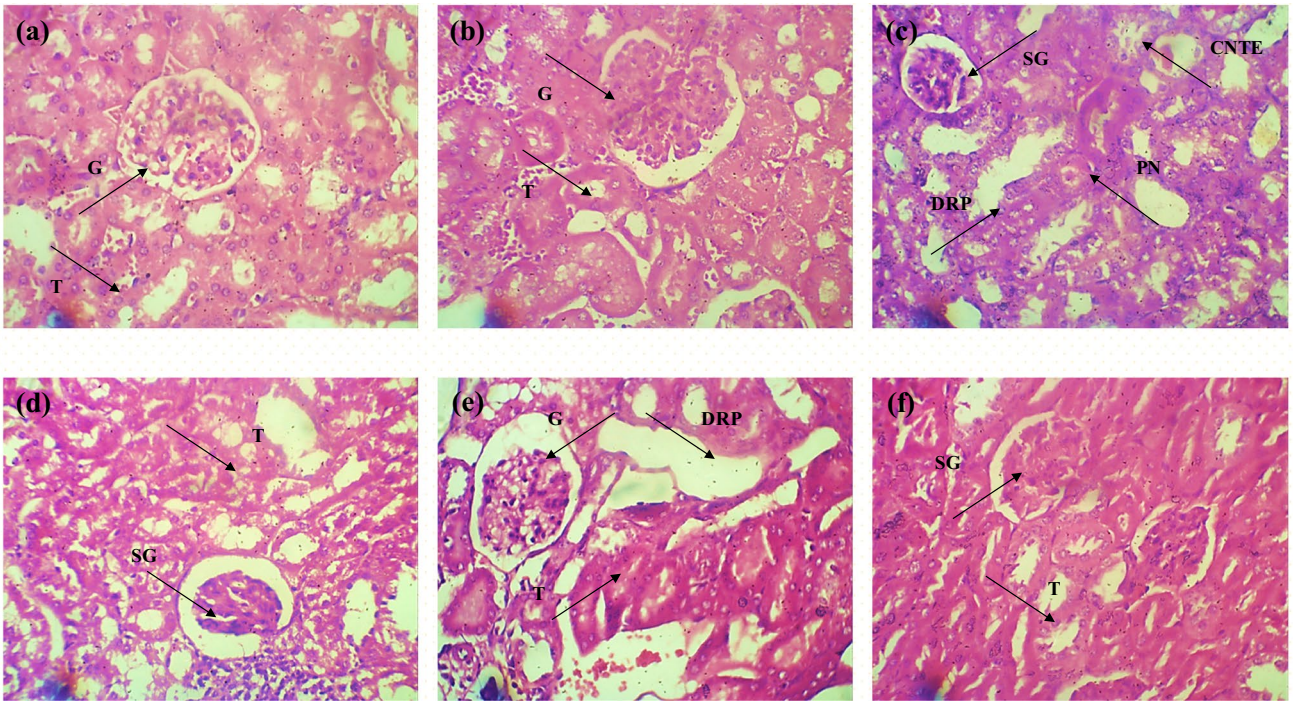

Fig. 3 Illustration of histological alterations in liver tissues (H&E;X400) (**a**) normal kidney tissues, (**b**) *L.brevis* MG000874 group, (**c**) B: G: D-gal group, (**d**) A: Ascorbic Acid group,(**e**) BG: D-Gal + *L. brevis* MG000874,(**f**) AG: Ascorbic acid + D-Gal., showed glomerulus (G), tubules (T),degeneration of renal parenchyma (DRP), cellular necrosis of tubular epithelium(CNTE) and pykonotic nuclei (PN)

during fermentation. They have a great influence on human life and are commonly utilized in many industries, like poultry, livestock, and food production (Landete et al. 2017; Ramos et al. 2020). LAB strains isolated from different sources like plants and animals, compost, fermented substances, the gastrointestinal tract of animals, and silage may have antioxidant activity (Rezaei et al. 2020; Unban et al. 2021). This trait is strain and species-specific. Therefore, the study aimed to investigate the antioxidant potential of the *L. brevis* MG000874 strain of LABs isolated from the animal (Asan et al. 2022), in the D-gal-stressed mice.

The constant administration of D-gal injections simulates and induces the OS by increasing the production of the FROS and depletion of the antioxidant activity (Li et al. 2015). The stress-affiliated mechanisms may be damaged by the unusual production of ROS (Ge et al. 2021); thus, the current research designated the D-gal-induced OS in mice to find the effects of the *L. brevis* MG000874 as an antioxidant against stress-related gene expression in the liver and kidney (Li et al. 2020). In our previous study, we injected 150 mg/kg/body weight D-gal in mice for inducing OS at 8 weeks (Noureen et al. 2019). In the current study, we selected the high dose of D-gal 300 mg/kg/BW for a quicker result. At this dose, the aging sign and symptoms appeared within 4 weeks in the mouse model. Important and basic indicators in biomedical research for observing the stress effects are the organ index in mice. In the stressed body, atrophy of the liver is more noticeable (Xu et al. 2016). An immune organ of an animal is the liver and its atrophy may be a source of immune deficiency (Chen et al. 2020). The literature revealed that the liver index of OS mice was considerably decreased as compared to the normal mouse liver (Sang et al. 2017). The metabolic organ is the kidney, and a decrease in kidney index has a great influence on metabolic ability (Xu et al. 2018). Thus, alterations in the organization of mouse organs can be noted in the organ index and were significantly important for evaluating the successful induction of OS in mice (Sang et al. 2017; Shen et al. 2011). The outcomes of this study presented that D-gal caused the atrophy of organs and induction of OS in mice (Woo et al. 2014; Qiu et al. 2017). OS processing was efficiently postponed by treating with *L. brevis* MG000874 (10^9 CFU/kg), proposing the noticeable anti-stress effect of *L. brevis* MG000874.

Histopathological inspection is the microscopic study of the cells, tissues, or organ morphology to verify the body changes (Di Meo et al. 2016; Qian et al. 2018). D-gal-induced OS mice revealed that some changes in liver and kidney tissues in comparison to the normal group were noticed. In the liver, corrupted hepatocytes with nucleus and nucleolus, high vascular degeneration in sinusoidal spaces with a hazy appearance, and uneven cell margin with messy cells were noticed as well. While in treatment groups, mice revealed a normal appearance of hepatocytes with a nucleus, clear radiating hepatic cells, stable

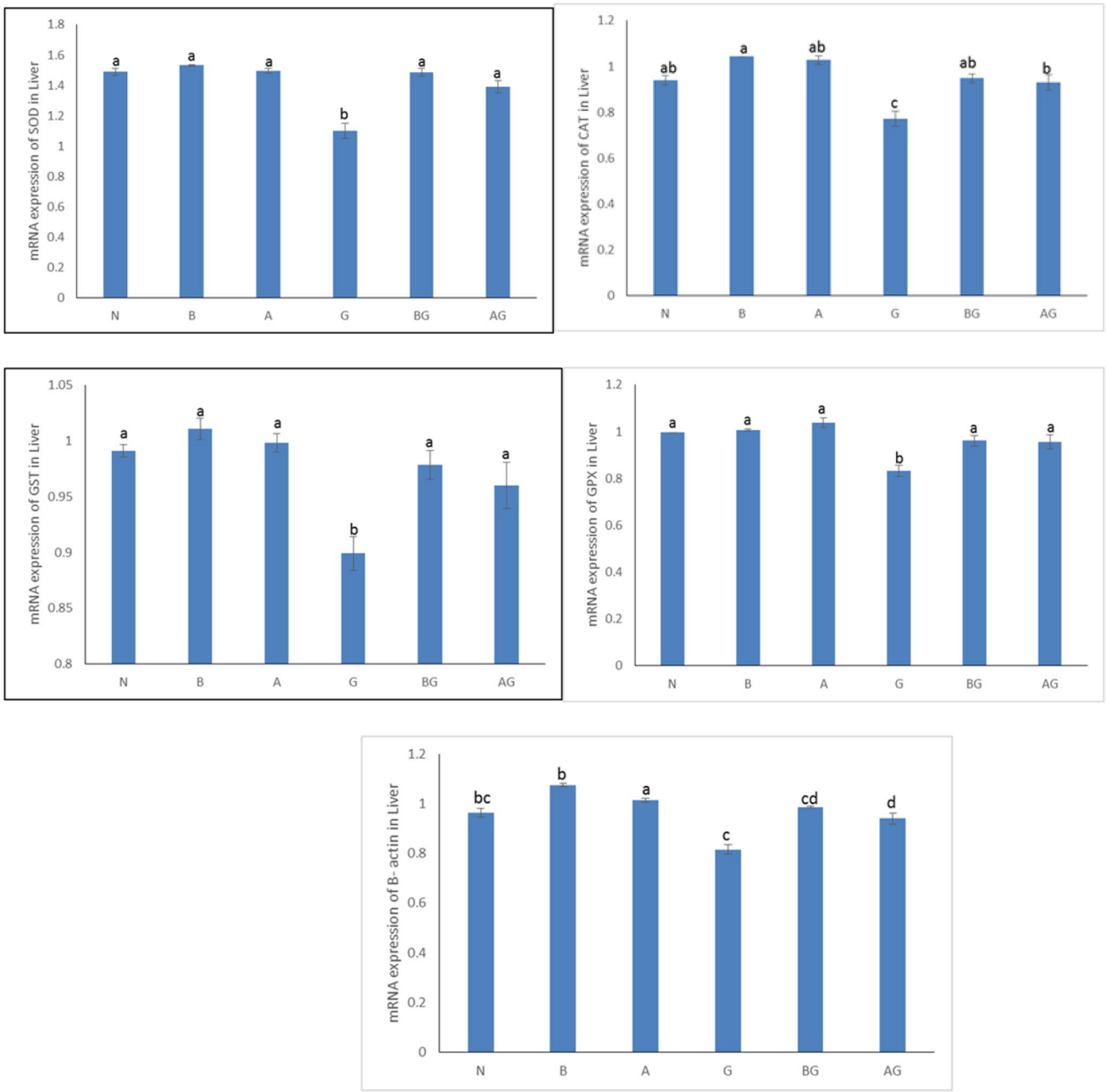

Fig. 4 Effects of *L. brevis* MG000874and ascorbic acid on D-Gal induced changes on the gene expression level of SOD,CAT, GST, GPX and B- actin in kidney. N: control; G: d-gal; B: *L. brevis* MG000874; A: Ascorbic Acid; BG: group getting both D-Galand *L. brevis* MG000874; AG: Groupgetting Ascorbic acid and D-Gal. Data represent Mean ± SEM; means withdifferent superscript letter (a, b, c, d) exhibits significant differences ($P < 0.05$).

interlobular bile duct, regular portal triad, and usual endothelial cells (Fig. 2e). In the treatment of *L. brevis* MG000874 (B) or ascorbic acid (A), the morphological abnormalities of the liver and kidney tissues were considerably upgraded, and the upgrading impact of *L. brevis* MG000874 was the most noticeable in the liver. The liver tissue morphology of the *L. brevis* MG000874–treated group was very close to that of group N. In the kidney, Bowman's capsule of N and B groups had regular inner podocyte cellular layer, outer squamous capsular cells, normal renal parenchyma, vascular pole, and proximal–distal convoluted tubules (Fig. 3c). Renal parenchyma of the D-gal group deteriorated, and tubular epithelial cells of the pyknotic nuclei were observed. These histopathological changes supported the results of antioxidant enzyme activity.

During the process of bio-oxidation, a huge quantity of ROS is generated in the body. The stability of ROS is attained by numerous antioxidant defense systems, containing, SOD, CAT, GST, GSH, and vitamins (Claiborne

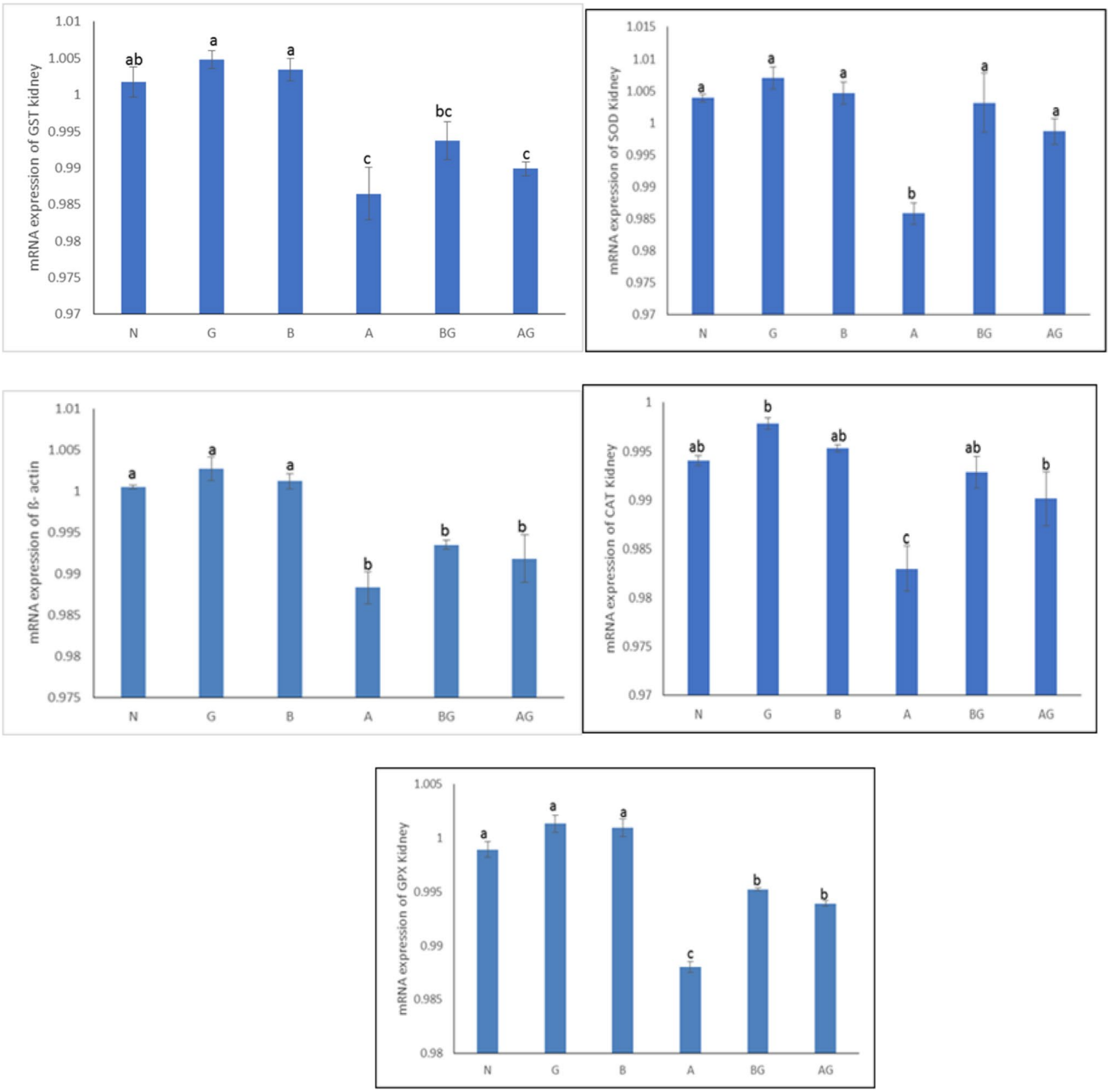

Fig. 5 Effects of *L. brevis* MG000874and ascorbic acid on D-Gal induced changes on the gene expression level of SOD,CAT, GST, GPX and B- actin in liver. N: control; G: d-gal; B: *L. brevis* MG000874; A: Ascorbic Acid; BG: group getting both D-Galand *L. brevis* MG000874; AG: Groupgetting Ascorbic acid and D-Gal. Data represent Mean ± SEM; means withdifferent superscript letter (a, b, c, d) exhibits significant differences ($P < 0.05$)

2018; Germoush et al. 2022). Their synergistic influence transforms excessive ROS into O_2 and H_2O_2 molecules (Motataianu et al. 2022). LABs have a specific antioxidant ability for quenching free radicals, which can support the antioxidant enzymes. Antioxidant activity is a strain-specific feature. And the body's metabolic process, LABs prevent the oxidation process by discharging antioxidant enzymes such as SOD (Feng et al. 2016). In this study, the *L. brevis* MG000874 significantly elevated the SOD activity and CAT activity in the kidney and liver tissue of the treated mice. The experimental outcomes confirmed that the induction of D-gal causes cellular OS and results in decreased SOD and CAT activity in liver and kidney tissues. Ascorbic acid or *L. brevis* MG000874 was good in the antioxidant effect during the measurement of SOD and CAT stress indicators in tissues (Di Meo et al. 2016).

To further enlighten the antioxidants, this research has observed the changes in SOD, CAT, GST, GPX, and B-actin expression levels. In animals, SOD is universally expressed as a mitochondrial antioxidant enzyme and helps in controlling free radicals' production by keeping healthy (Zhao et al. 2018). CAT functions as scavenging O-free radicals and stimulating the disintegration of H_2O_2 by preventing oxidation damage (Selvaratnam and Robaire 2016). The literature revealed that OS causes tissue atrophy by dropping SOD and CAT expression (Hart et al. 2015; Hassani et al. 2018). The outcomes of this study revealed that mRNA expression levels of SOD and CAT in the liver and kidney tissues of the D-gal control group were significantly reduced ($p < 0.05$), representing that D-gal subcutaneous injection in mice may cause OS. Though, mRNA expression levels were significantly improved in treatment with *L. brevis* MG000874 ($p < 0.05$). GST is a broad-spectrum antioxidant. GPx may be used to regulate the intracellular hydroperoxide level in the gastrointestinal tract (Sharma et al. 2021). ß-actin is the main structural protein of the cytoskeleton, which plays a significant part in retaining the shape of the cell (El-Sayed et al. 2021). We found that GST, GPX, and ß-actin expression levels were upregulated positively by *L. brevis* MG000874 in D-gal-induced model mice as compared to the G group. These reports indicated that *L. brevis* MG000874 could perform as an antioxidant, which may directly hunt ROS.

Finally, histological investigation verified that cellular inflammation and apoptosis of hepatocytes were greater in the D-gal alone group, and treatment with *L. brevis* MG000874 showed normal hepatocytes with congested sinusoids in the liver. While in the kidney, the disintegration of renal parenchyma, some cellular necrosis, and the vanishing of tubules spaces were identified in the stress group as compared to group BG. *L. brevis* MG000874 (probiotic) possessed a renal, hepatic protective influence through raising activities of antioxidant enzymes (i.e., SOD, CAT) on D-gal-inducted stress (BG) and maintaining the cellular structure. These consequences were also connected to the stimulation of the antioxidant defence system by boosting SOD, CAT, GST, GPX, and ß-actin controlled gene expression. Our findings further proposed that *L. brevis* MG000874 could maintain the intracellular redox balance in the livers and kidneys of D-gal-treated mice by renewing the activities of antioxidant enzymes.

Conclusion

The current investigation found that *L. brevis* MG000874 has an antioxidant effect. Induction of D-gal-initiated OS and reduced the antioxidant activity by down-regulating the mRNA expression of different antioxidant enzymes. This down-regulation depends on the variability and different tissue responses. Supplementation of *L. brevis* MG000874 or ascorbic acid resulted in body weight gain, and organ index restored the antioxidant defence system by improving the antioxidant enzyme status of SOD and CAT and up-regulating the gene expression of SOD, CAT, GST, GPX, and ß-actin. As a whole, these findings verified that *L. brevis* MG000874 promoted the antioxidative gene expression and cellular antioxidative responses in vivo. Therefore, *L. brevis* MG000874 would have the potential to be further explored as an antioxidant functional food in the prevention of more stress-related diseases.

Author contribution S. N: conceptualization, methodology, analysis, data curation, and writing — original draft; T. H: Analysis; A. N: reviewing and editing; A. E. A: reviewing and editing.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate The protocol for these experiments was approved by the Animal Ethics Committee of the Virtual University of Pakistan.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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