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Article - Human and Animal Health

Evaluation of *Lactobacillus brevis* MG000874 in Behavioral and *In Vitro* Antioxidant Enzyme Activity of Murine Brain

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HIGHLIGHTS

- Oxidative stress is considered as a major factor that accelerates the aging process.
- D-Galactose impairs ambulation, rearing, leaning and grooming responses in mice.
- *L. brevis* MG000874 can improve cognitive responses and levels of antioxidants.
- *L. brevis* MG000874 can be further confirmed as anti-aging and antioxidant agent.
- It is recommended to include *L. brevis* MG000874 in the list of probiotics.

Abstract: Aging and oxidative stress are important biological processes with extensive medical and social significance. The D-Galactose (D-Gal) has been used in animals for inducing oxidative stress and brain aging. The present study was conducted to evaluate the role of *Lactobacillus brevis* MG000874 in inhibiting the aging process and oxidative stress in D-Gal induced oxidative stress murine model. The cognitive responses and biochemical indicators were used to estimate the anti-aging and antioxidant potential of *L. brevis* MG000874 in mice. Tests used to assess cognitive responses, included object recognition, open field and T Maze. While antioxidant status was assessed by measuring superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione-S-transferase (GST) activity in mice brain tissues. Results suggested that D-Gal can seriously impair the object recognition in mice. It led to significant reduction in ambulation, rearing, leaning and grooming. Similar trend was observed in antioxidant enzymes such as SOD,

CAT and GSH except for GST where elevation was observed. Treatment with *L. brevis* MG000874 and ascorbic acid inhibited this reduction and kept levels close to control. Likewise, values of GSH of probiotic group were lower than negative control but significantly higher than positive control group. Probiotic supplementation in the presence of D-Gal displayed improvement in GSH, while no such improvement could be observed with ascorbic acid treatment. It can be concluded from the present study that *L. brevis* MG000874 can be used as anti-aging and antioxidant agent. It is recommended that *L. brevis* MG000874 could be included in the list of probiotics.

Keywords: *L. brevis* MG000874; D-Galactose; anti-aging and antioxidant potential; probiotics.

INTRODUCTION

Aging is defined as time dependent functional and physiological decline phase in which the cells of body break down resulting in slow renewal and increasing susceptibility to diseases [1,2]. The aged brain may be more vulnerable to stress and deficit in memory. Furthermore, previous studies have shown that aging causes decrease in efficiency of working memory and limit its processing to perform any task [3,4]. Researchers have a great interest in understanding, preventing, and treating the abnormal aging processes.

D-Galactose (D-Gal) is a monosaccharide sugar and its normal quantities can be metabolized completely. Whereas, its higher concentration produces galactose oxidase, aldose and hydrogen peroxide which lead to production of superoxide anions. Production of free radicals such as reactive oxygen species (ROS) and peroxides are mainly destructive feature of oxidative stress and cause damage to biomolecules including lipid, proteins and nucleic acids [5, 6]. It is well known that continuous exposure to D-Gal speeds up aging in rats, mice and drosophila [7]. Further evidence has shown that long term exposure of D-Gal induces aging through formation of ROS and elevation of oxidative stress in mice ([7]. Decreasing ROS and free radical formation might provide a possible solution for aging-related disorders.

Antioxidant enzymes have been reported to have neuroprotective and neuroregenerative effects through reducing or reversing cellular damage [8]. The antioxidant repair system such as catalase and superoxide dimutase (SOD) exist in the body but sometimes these systems are not much efficient to protect the body from oxidative damage. Hence, consuming antioxidant supplements is thought to prevent body from oxidative stress.

Probiotic bacteria are health promoting microbes and are mainly present in fermented dairy products such as yogurt, cheese, butter and pickles [9]. Mostly probiotic bacteria belong to Lactic acid bacteria (LAB) such as *Lactobacillus* sp. and *Bifidobactria* sp. [10]. LAB are known to produce extracellular metabolites which are distinguished by their ability to eliminate ROS [11]. These metabolites help in reduction of lipid peroxidation and regulation of non-enzymatic and enzymatic antioxidants [5, 12]. Several LAB strains have been reported to have antiaging effects that might be due to their radical scavenging activity and enhancement effect of antioxidant enzymes, however, their efficiency is strain specific [13]. Owing to the antioxidant property of LAB strains, they have got special attention of scientists and are explored for their safe use as natural antioxidants.

In our previous study, we published the ameliorating role of *L. brevis* MG000874 in liver, kidney and serum [14]. This study was aimed to analyze the ameliorative effect of *L. brevis* MG000874 supplementation on the memory, exploratory behavior and anxiety through cognitive responses and enzymatic and nonenzymatic antioxidant levels in D-Gal induced aging mice model. SOD, CAT, glutathione-S-transferase (GST) and glutathione (GSH) activity was measured to assess the oxidative stress in murine brain.

MATERIAL AND METHODS

Animals

This study was approved by Institutional bioethics committee vide letter no. 3456/08/20 dated 25/09/2020 following procedures approved by committee for control and supervision of experimental animals, Pakistan. A total of 60 albino mice (*Mus musculus*, 8 weeks old, 27 ± 2 g) were kept in cages under controlled conditions (Temperature 22 ± 2°C; humidity 45 ± 5%, 12 h light/dark cycles). Free access to food and water was provided during the experiments. Mice were allowed to acclimatize for one week prior to experimentation.

Experimental groups

The mice were divided randomly in to 6 groups: Group I was kept as negative control (CG), Group II was treated with D-Gal and maintained as positive control (GG), Group III was treated with ascorbic acid (AG), Group IV was given *L. brevis* MG000874 intact cells (PG), Group V was treated with D-Gal and *L. brevis* MG000874 intact cells (PGG) and Group VI was given D-Gal and ascorbic acid (AGG). Animal grouping and treatments are summarized in Table 1. Treatments continued for duration of 8 weeks.

Table 1. Different treatments of the experimental groups for a period of 8 weeks.

Sr#	Treatments*	Groups (n=10)**					
		I	II	III	IV	V	VI
		CG	GG	PG	AG	PGG	AGG
1	Normal saline (Subcut injection)	+	-	+	+	-	-
2	D-Gal (150mg/kg BW) (Subcut injection)	-	+	-	-	+	+
3	*L. brevis* MG000874 (~10^{10} CFU/mL) (feeding tube)	-	-	+	-	+	-
4	Ascorbic Acid (5mg/animal) (feeding tube)	-	-	-	+	-	+
5	Normal saline (feeding tube)	+	+	-	-	-	-
6	Sampling (at 60day)	+	+	+	+	+	+

* Normal saline 0.2mL (0.89%), Ascorbic acid 0.2mL of 25mg/mL solution (5mg/mouse), D-Gal 0.2mL (150 mg/mouse). Intact cell 0.2mL/animal/day (~10^{10} CFU/mL). ** CG: negative control group, GG: D-Gal treatment group (positive control), AG: ascorbic acid treatment group, PG: intact cell (*L. brevis* MG000874) treatment group, PGG: D-Gal and intact cell (*L. brevis* MG000874) treatment group, AGG: D-Gal and ascorbic acid treatment group. +: treatment administered, - : treatment not administered.

Pilot study

Pilot study was run to decide the time duration of exposure to D-Gal required for observing significant changes in cognitive responses of mice. A daily dose of (150mg/kg/day) of D-Gal was administered subcutaneously once for 60 days on the basis of pilot study (data not included).

Bacterial treatment

On the basis of previous literature on LAB probiotic species [14], 200 µL of ~10^{10} CFU/mL bacterial density of *L. brevis* MG000974 was decided to administer to each mouse per day through oral route using intragastric gavage.

Study design

Behavioral impairments are results of age-associated neurodegeneration, therefore behavioral test were used as indicators of the aging. For this reason, decreased performances in exploratory tests are taken as markers of aging [15]. The leaning, memory and cognitive behaviors were compared among groups using object recognition and open field test.

Object recognition test

Object recognition test is a memory consolidation test from which the obvious object memory was assessed. In this test, after familiarization of two identical objects, mice are presented with an old and a new object, mice remembering the old object spend more time investigating the new one.

The examination area (40 × 30 × 20 cm) was created from wood. The apparatus was placed in a room with dim light by a 40 lux. The objects used were three replicates of cuboid green plastic blocks (A; 4 × 4 × 5 cm) and white cylinder plastic bottle (B; 7× 4 cm) filled with water. These objects were heavy so that the mice couldn't displace them.

The test was performed for three consecutive days. On day 1, mice were placed individually, in the area for 10 minutes for habituation. On day 2, two identical objects (O1, O2) were kept on the both sides. The distance of the object with wall was 8 cm while between objects was 16 cm. Later on, each mouse was left in the center of the two objects and allowed to explore the area for 10 minutes and data was recorded. On day 3, one formerly observed object and one novel object were placed there in the field (FO, NFO) and the

test was repeated and data was recorded for 10 minutes. Object exploration was taken as the direction of the animal's nose close the object within 2 cm or less. The time recorded for exploration was used to calculate memory discrimination index (DI). DI = (*N* − *F*)/ (*N* + *F*), where *N* is time spent exploring the new object and *F* is time spent exploring the familiar object. Higher DI means that memory holding is good for the previous object.

Open field test

Mice locomotor activity and anxiety was evaluated using open field test. Animals were placed in an open field box. The open field box was a square field (50 cm) built from wood and there was light bulb above the center making it bright. The floor of field was divided into 16 gridlines by white tape. Test was executed from 8:00 to 15:00 h. Each mouse was placed in the center of the field. Adaptation time was for 1 minute after which mice behavior was noted for five minutes. Open field was cleaned with a slightly moist cloth each time. The behavioral parameters included (1) ambulation: the number of lines crossed in the area during the observation time; (2) rearing: the number of times the mouse remains on its rear legs; (3) leaning: the number of times the mouse placed one or two forelimbs on the wall of the field; (4) grooming: the number of times the mouse cleans itself combing or scratching of any part of the body.

T-maze test

T- maze test was performed to study rodent memory and spatial leaning. The elevated T-maze was made of thermophore sheet with three arms of equal dimensions (30 × 16 cm). It was the shape of T with one arm (enclosed by walls 16 cm high) perpendicular to two opposed arms. Plexiglas frame 0.5 cm high enclosed the open arms. This apparatus was raised up 40 cm above the floor. The trials were executed with mild background music.

After 15 minutes of acclimation in the experimental room, the mice were placed at the border of enclosed arm and time of appearance was recorded from this arm to all four paws (baseline latency). Baseline latency is probably the motor activity efficiency of mice. This procedure was repeated in two succeeding trials (Inhibitory Avoidance 1 and Inhibitory Avoidance 2) with interval of one minute. After avoidance 2 the mice were placed at the edge of an open arm and the time was recorded to enter into closed arm (escape 1). After 72 hours, the avoidance (inhibitory avoidance 3) and escape (escape 2) latencies were recorded again. Avoidance 3 was considered as measure of memory and anxiety. Mice prefer to stay in a place where they have already been exposed. The reduction in time spent in previously exposed place was considered as indicator of stress and short of memory. The escape from the open arm of the maze was considered as species-typical fear of openness and elevation. The model was considered established when an increase in escape response was observed in D-Gal group.

Biochemical Assays

Tissue homogenate preparation

After slaughtering, the brain tissue was removed and washed twice with saline to remove blood and other contaminants. After washing, 0.1g of brain was weighed and suspended in 1 mL of PBS (Invitrogen) buffer (pH 7.4) to prepare its homogenate using potter-elvehjem homogenizer under chilled condition. The homogenate was centrifuged at 6,000×g for 10min at 4°C using a refrigerated centrifuge (Sigma, 2K15) and supernatant was collected. The supernatants from all animals were stored at -80 ℃ to proceed further. The levels of antioxidants including SOD, CAT, GST and GSH (Sigma-Aldrich) were estimated in brain supernatant following Marklund and Marklund, [16], Claiborne [17], Habig and coauthors [18] and Jollow and coauthors [19], respectively, while activity was calculated as per gram of wet tissue weight. The detailed procedure of above parameter has been described in previous study [14].

Statistical analysis

The statistical analyses were performed using the SPSS software, Version 11.5. Data was analyzed by one way ANOVA and Kruskal Wallis test. Data was expressed as Means± SE of mean. Statistical significance was fixed at $p < 0.05$.

RESULTS

Pilot study

Pilot study was conducted to investigate the effect of D-Gal treated aging mice on the memory impairment compared to negative control using behavioral tests.

Object recognition test

Comparison between discrimination index (DI) of negative control (CG) and D-Gal group (GG) after one and two months duration are presented in Figure 1. One month treatment with D-Gal slightly affected the object recognition ability of mice (p >0.05). However, after two months of exposure with D-Gal, discrimination index (DI) depleted significantly, (0.19 ± 0.02 VS 0.43 ± 0.04) in D-Gal treated group (p < 0.05).

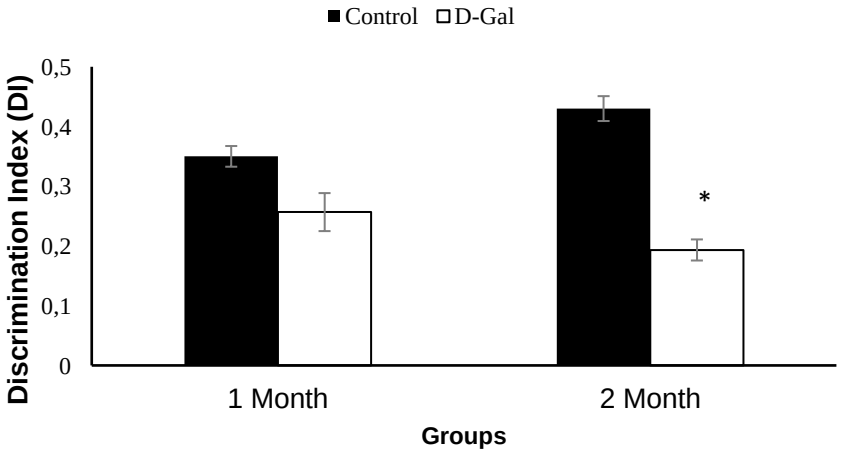

Figure 1. Comparison of discrimination index (DI) after one and two months of exposure to D-Gal. All values are expressed as Mean ± SEM using independent sample *t'* test. Asterisk on bar show significant differences at $p \leq 0.05$.

Open field Test

In open field test, four parameters *viz*., ambulation, rearing, leaning and grooming were recorded. The results were compared between control and D-Gal group. One month treatment with D-Gal slightly affected the ambulation, a locomotor activity of mice but it was not statistically significant compared to control (p > 0.05). However, two months exposure to D-Gal further depleted ambulation to 45.2 ± 4.005 which was significantly lower compared to its respective control group (100.6 ± 16.302) (p < 0.05) (Figure 2A). Similar trend was recorded in leaning activity i.e., after one month of treatment, slight reduction was observed from 23.4 ± 3.89 to 19.6 ± 1.76 compared to control in D-Gal treated group. However, two months exposure to DGal further depleted leaning activity which was significantly lower compared with its respective control group from 24.4 ± 3.87 vs 8.2 ± 1.80 (T, P) (Figure 2B). D-Gal treatment impaired the rearing activity in mice significantly from 7.4 ± 3.17 to 4.8 ± 1.32 after one and two months exposure respectively. Whereas, in control group, no significant change in rearing activity was observed after one and two months (Figure 2C). The grooming of control group after one and two months was recorded as 9.4 ± 1.03, 15.6 ± 1.50 while in oxidative stress group, it was 8 ± 1.64 and 2 ± 0.45 respectively. D-Gal depleted the grooming up to 2 ± 0.45 which was significantly lower compared with its respective control group (p < 0.05) (Figure 2D).

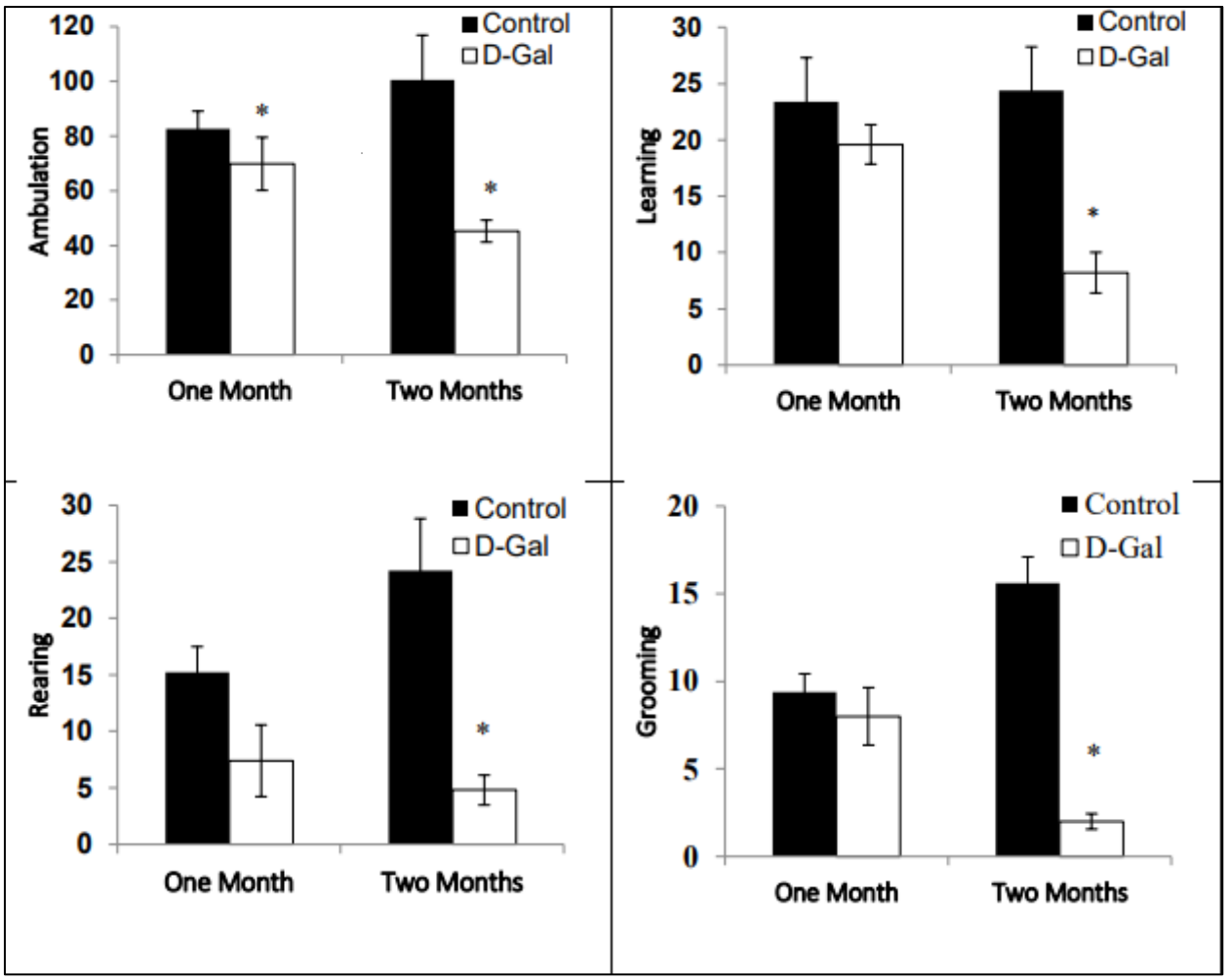

Figure 2. Establishment of Experimental Model of Oxidative Stress: Effects of D-Gal treatment on Ambulation (A), Leaning (B), Rearing (C) and Grooming (D). Bars represent Mean ± SEM. Data were compared using independent sample *t'* test. Asterisk on bar show significant difference from respective control at $p \leq 0.05$.

T-Maze Test

D-Gal treatment impaired the long term memory in mice. One month treatment with D-Gal showed that the avoidance latency along trial was increased in both GG and control. No significant difference could be recorded in Avoidance 1 and 2 but avoidance 3 was significantly ($p < 0.05$) higher in Control group ($p < 0.05$), after both one and two month exposure to D-Gal (Figure 3).

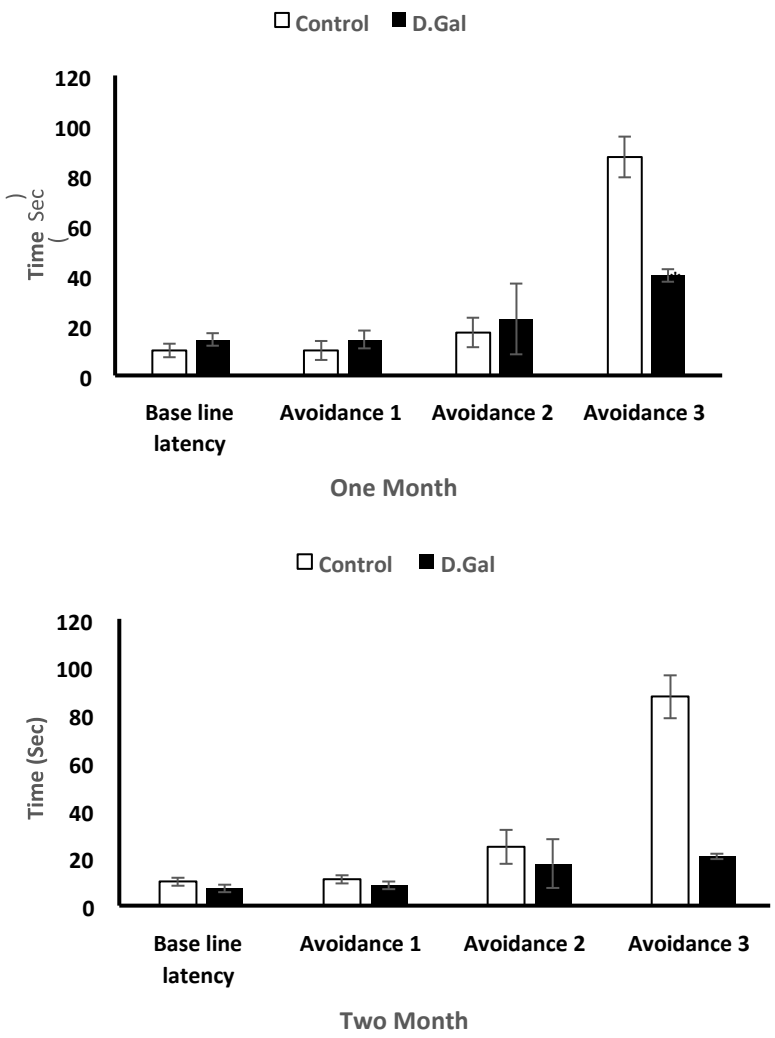

Figure 3. Establishment of d-gal induced brain aging model: Effects of d-Gal on Effects of D-Gal treatment on Base line latency, Avoidance 1, 2 and 3 following one and two month exposure to D-Gal. Bars represent Mean ± SEM. Comparison was made using independent sample t-test. Asterisk indicate significant difference from respective control at $p < 0.05$. Main study

Object recognition test

The impairment of object novelty preference among mice groups with different treatments are shown in Figure 3. The comparison of discrimination index indicated depletion in DI (0.19 ± 0.02) of object recognition test in D-Gal induced oxidative stress group. Ascorbic acid treatment had intermediate level of DI (0.30 ± 0.06) but it was not significantly different from control group as well as from oxidative stress group. However, animals with probiotic supplementation showed high level of DI (0.37 ± 0.02) which was significantly better than D-Gal group (Figure 3).

In the main trial, open field test results indicated that the oxidative stress led to reduction in ambulation (45.2 ± 8.95). D-Gal group (GG) was significantly different from control group (100.60 ± 36.45) whereas, all other groups were close to control. D-Gal treated group displayed significant reduction in rearing (4.8 ± 2.94) compared to control group (24.2 ± 10.35). However, treatment with *L. brevis* and ascorbic acid inhibited this reduction and kept level close to control. Similarly, exposure to D-Gal significantly reduced leaning (8.2 ± 4.02) compared to control (24.4 ± 8.64) ($p < 0.05$). Although, no significant difference was observed among all groups compared to control. All groups showed similar result as control but D-Gal group displayed significant reduction in grooming (2 ± 1.00) compared to control group (15.6 ± 3.36) ($p < 0.05$).

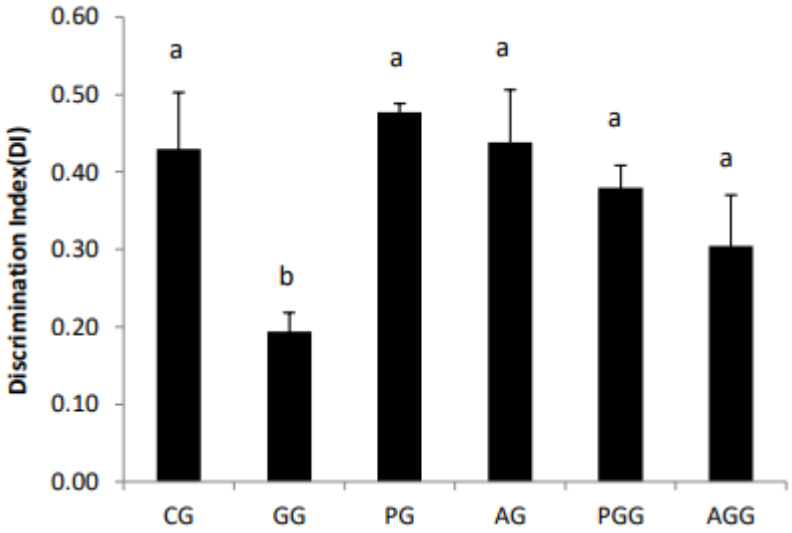

Figure 4. Comparison of discrimination index (DI) among different groups. CG, negative control group, GG: Positive control group, PG: probiotic group, AG: Ascorbic Acid group, PGG, group receiving both D-Gal and Probiotic, AGG: Group receiving Ascorbic acid and D-Gal. Data are presented as Mean ±SE, Data were analysed using one way ANOVA followed by DMRT, bars different letters show sig nificant differences at $p < 0.05$.

Open field test

Similar to pilot trial, the D-Gal exposure led to reduction in ambulation, rearing, leaning and grooming but these changes were not observed in animals which received *L*. *brevis* MG000874 or ascorbic acid alone or along with D-Gal (Figure 5).

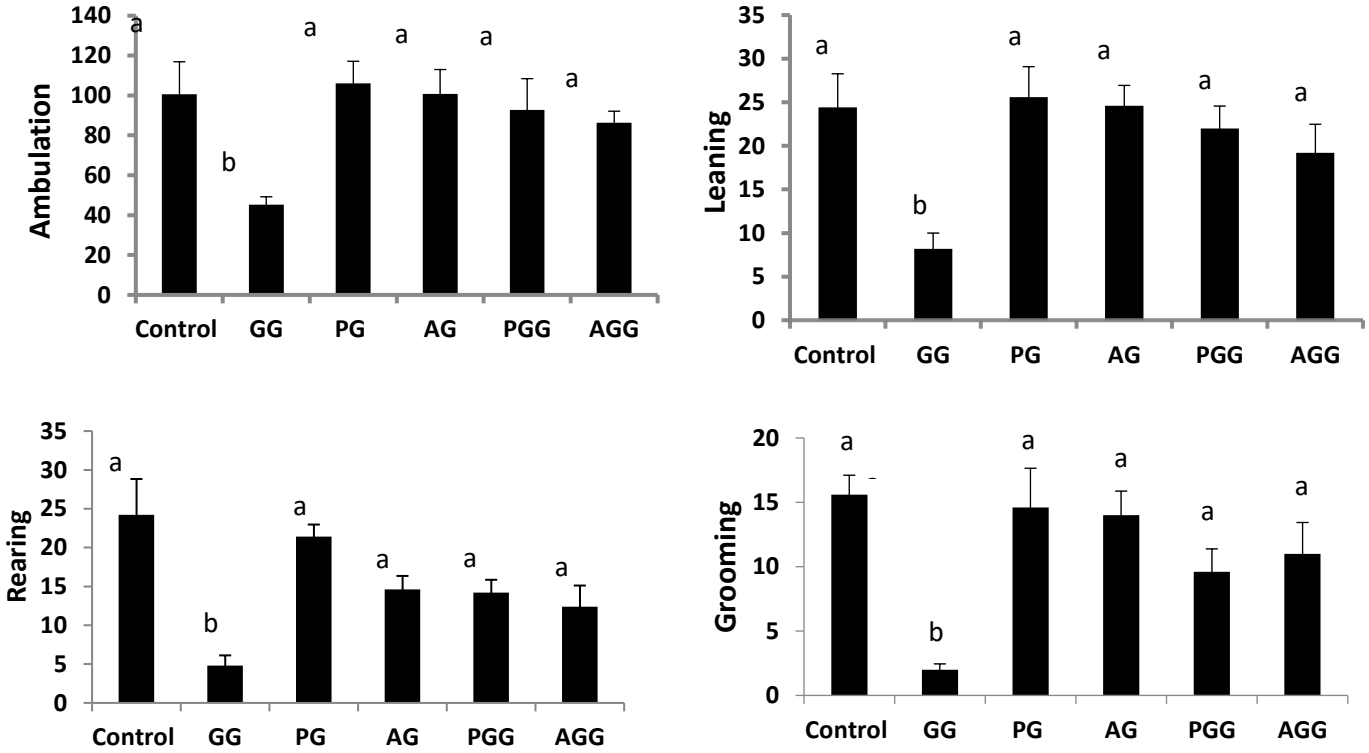

Figure 5. Preventive effects of *L*. *brevis* MG000874 and ascorbic acid against D-Gal induced changes in ambulation, leaning, rearing and grooming responses of mice. GG: Positive control group, PG: Probiotic group, AG: Ascorbic Acid group, PGG: group receiving both D-Gal and Probiotic, AGG: Group receiving Ascorbic acid and D-Gal. Data were analyzed using one-way ANOVA followed by DMRT, bars different letters show significant differences at $p < 0.05$.

T-Maze Test

In escape latency was calculated by finding difference between Escape- 1 and Escape-2. Data indicate that was no significant difference (p value of F = 0.259) between different treatment groups, but animals of positive control group took more time in going to dark. However, significant variation was observed in Avoidance 3. The values of treated groups were much improved and better than positive control and differences were statistically significant (Figure 6).

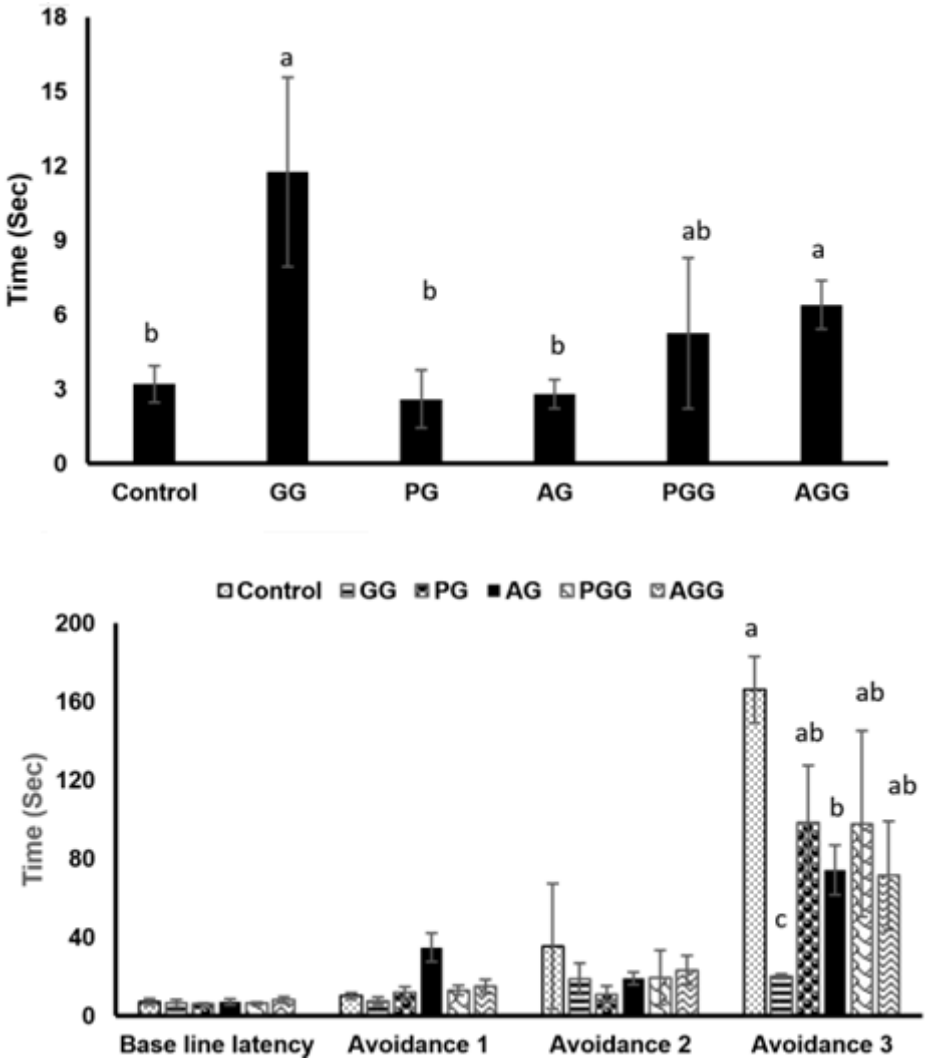

Figure 6. Effects of *L*. *brevis* MG000874 and ascorbic acid on D-Gal induced changes in Escape response, base line latency, Avoiance 1-3 in mice. GG: Positive control group, PG: Probiotic group, AG: Ascorbic Acid group, PGG: group receiving both D-Gal and Probiotic, AGG: Group receiving Ascorbic acid and D-Gal. Data were analyzed using one-way ANOVA followed by DMRT, bars with different letters show significant differences at $p < 0.05$.

Antioxidant Enzymes

D-Gal treatment led to reduction in SOD, CAT and GSH while elevation in GST activity. LAB and ascorbic supplementation could resist the change in CAT and CAT values in probiotic (PG) and ascorbic acid (AG) treated groups were comparable with negative control. The GSH values of probiotic (PG) group were still lower than negative control but significantly higher than positive control (GG) group (p <0.05). SOD values of probiotic (PG) group showed significant increase ($p < 0.05$) compared to positive control (GG). Likewise, supplementation of LAB in the presence of D-Gal displayed improvement in GST, while no such improvement could be observed with ascorbic acid (AG) treatment group (Table 2).

Table 2. Effect of different treatment on antioxidant enzymes.

Groups	SOD (U/g)	CAT (U/min/g)	GST (nmol/min/mL)	GSH (µMGSH/g)
Control (CG)	407.2 ± 2.06^{e}	18.04 ± 2.33	36.27 ± 1.85^{b}	528.67 ± 1.74^{d}
D-Gal treatment (GG)	117.37 ± 1.42^{a}	10.33 ± 1.42	63.24 ± 2.07^{c}	269.63 ± 1.88^{a}
Probiotic treatment (PG)	444.07± 2.12^{d}	15.05 ± 1.74	30.23 ±1.01^{a}	493.02 ± 1.85^{d}
Ascorbic acid treatment (AG)	335.18± 2.60^{f}	17.20 ± 1.69	23.92 ± 1.72^{ab}	530.02 ± 2.88^{c}
Probiotic + D-Gal (PGG)	273.34 ± 1.70^{c}	15.16 ± 1.64	26.56 ± 1.46^{a}	318.4 ± 2.36^{b}
Ascorbic Acid+ Stress (AGG)	246.64 ± 1.45^{b}	12.66 ± 1.78	27.22 ± 1.80^{a}	264.5 ± 3.01^{c}

Data represent Mean ± SEM. Values having different letter (in column) are significantly different at $p < 0.05$. ** CG: negative control group, GG: D-Gal treatment group (positive control), AG: ascorbic acid treatment group, PG: intact cell (*L. brevis* MG000874) treatment group, PGG: D-Gal and intact cell (*L. brevis* MG000874) treatment group, AGG: D-Gal and ascorbic acid treatment group.

DISCUSSION

Aging is an inevitable biological process of growing older, which is accompanied by signs of decline in the cognitive function, adaptability and immunity which inturn is an important risk factor for many metabolic disorders, including hypertension, type 2 diabetes, atherosclerosis, and senile dementia, whereas the onset of these diseases can be delayed by slowing down the aging process [20, 21]. Molecular and genetic biologists are trying hard to find out an actual remedy against aging. Aging is the result of neurotoxicity caused by oxidative stress [22].

D-Gal has been used in many studies to induce aging in rodents. In current study, D-Gal treatment (150mg/kg/day for 8 weeks) led to great impairment in the memory of mice. One month treatment with D-Gal slightly affected the recognition ability of mice ($p > 0.05$) But after two months continuous exposure to D-Gal significantly depleted discrimination index (DI), the indicator of memory. These findings are consistent with the work of Wei and coauthors [23] and Pourmemar and coauthors [7] that D-Gal can bring noteworthy leaning and memory impairment in mice.

In open field test, D-Gal treatment greatly impaired activities of ambulation, rearing, leaning and grooming. One month treatment with D-Gal, at a dose of 150 mg/kg/day, slightly affected the exploration activity of mice however two months exposure to D-Gal further depleted ambulation and leaning/rearing which was significantly lower compared with its respective control group (p <0.05). Similar results were reported by Lu and coauthors [24] and Lu and coauthors [25] following exposure to D-Gal at a dose of 50 mg/kg/day through intraperitoneal injection and further observed that D-Gal treatment differently affects the brain/behavioural indicators of anxiety or fear. This could be due to difference in environment and other laboratory conditions and animal species.

D-Gal treatment also impaired the long term memory and slowed down locomotary responses in mice, noticed in elevated T-maze test. One month treatment with D-Gal showed that the avoidance latency along trial was increased in both D-Gal and control group but it was slightly higher in control. But after two months of exposure, avoidance latency recorded in D-Gal and control group was statistically significant on third trial. These results are in agreement with Chogtu and coauthors [26] and Tabrizian and coauthors [27]. The escape test performed on day one and five, showed that there D-Gal exposure slowed down locomotory activity. The significant differences between different groups were observed indicating that D-Gal treatment also affected escape latency in mouse. These results are similar to the findings of Chogtu and coauthors [26]. Probably DGal treatment differently affects the brain/behavioral indicators of anxiety or fear.

In main study, the results of pilot study were reproduced following D-Gal exposure, the data were recorded after 2 months treatment with either probiotic or ascorbic acid. Two groups PG and AG were used for in vivo safety assessment of probiotic, while groups PGG and AGG were run to check D-Gal stress ameliorating influence of probiotic. The stress led to depletion in DI of recognition test. Ascorbic acid treatment had intermediate level of DI but it was not significantly different from control group as well as from stress group (GG). Animals with probiotic supplementation shows high level of DI which was significantly better than D-Gal group. These findings suggest that probiotic supplementation might have helped in

decreasing the stress effects of D-Gal treatment. similar observations were reported by Savignac and coauthors [33], who indicated that enteric microbiota are helpful in decreasing anxiety and modulating memory processes in cognitive tasks.

Similarly, D-Gal induced stress led to reduction in locomotor activity ($p < 0.05$) of open field test. The performance of D-Gal group was significantly different from control group, probiotic as well as ascorbic acid group that support our hypothesis that probiotic supplementation may help in reducing induction of oxidative stress by D-Gal. Our findings are consistent with Scott and coauthors [34]. Authors observed that intestinal microbiota has significant association with age associated cognitive decline. Probably, *L. brevis* MG000874 given to D-Gal group had colonized the gut and became the part of intestinal microbiota and helped in reducing the oxidative stress induced by D-Gal group. We have recently reported production of antioxidant metabolites from probiotic strains including *L. brevis* MG000874 [35].

The stress led to reduction in avoidance observed in T-Maze test. In avoidance 3, D-Gal group showed significantly low avoidance ($p < 0.05$) with respective control, probiotic as well as ascorbic acid treated groups. Probiotic plus D-Gal group had reduced anxiety level in mice which is consistent with the work of Bravo and coauthors [36]. Authors studied the preventive effects of *L. rhamnosus* and suggested that there is essential role of probiotic bacteria in the communication of gut-brain axis.

Brain is mainly susceptible to oxidative harm due to its high oxygen demand, intense production of free radicals, and high level of transition metals such as iron, that catalyze the production of reactive oxygen species [28]. We have recently published the influence of *L. brevis* MG000874 antioxidant potential for various tissues in D-Gal oxidative stress model [14]. The current is focused on its influence on antioxidant status of brain tissue. with reference to negative control group the level of GSH remained significantly low in D-Gal group. The depletion in GSH level is a direct indicator of presence of the high amount of ROS production [29].

The level of SOD decreased significantly in the D-Gal group compared to control, which depict that in DGal group, SOD enzyme efficiently work to decrease the level of ROS formation. CAT converts the hydrogen peroxide into water and oxygen. In current study, D-Gal treated animal displayed significant reduction in SOD and CAT compared to control group, It might be due to degradation of enzyme due to D-Gal ROS or down regulation of SOD gene expression. This results are consistent with the previous reports by Wang and coauthors [30] and Zhang and coauthors [31], who used the same parameter for establishment of oxidative stress in different organs of mouse model. GST level in brain significantly increased in D-Gal group. Previously Shen and coauthors [32], reported that high level of GST in oxidative stress condition may be due to the formation of some metabolic products.

For safety assessment of strain, probiotic group was compared with control group in object recognition test, open field and T-maze test. Our strain showed least toxicityin mice and its value was near to control group. that the data clearly indicate that *L. brevis* MG000874 is safe tfor use in experimental studies. The values of indicators of oxidative stress indicators, SOD, CAT and GSH in brain were close to negative control which indicated that *L. brevis* is nontoxic and safe to use.

Collectively, in the current study, the probiotic strain *L. brevis* MG000874, was evidenced to possess antioxidant potential and indicate that it may play roles as antiaging agent, that efficiently revert the level of SOD, CAT and GSH levels in brain. Previously, antimicrobial potential of various probiotic isolates was also reported [37, 38]. These results corroborate with previous studies [39, 40]. However, actual mechanism involved in anti-aging process needs further experimentation.

CONCLUSION

In culmination, present study showed that probiotic *L. brevis* MG000874 can be used as anti-aging and antioxidant agent. Furthermore, *L. brevis* MG000874 supplementation shows positive impacts on behavior and cognitive function of mice that was evidenced in object recognition, open field and T-maze test. *L. brevis* MG000874 can improve antioxidant status of host. It is recommended that *L. brevis* MG000874 could be included in the list of probiotics. Further research is needed to understand mechanism involved in antiaging phenomenon.

Conflicts of Interest: Authors declare no conflict of interests.

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