Protective effect of Lactobacillus plantarum ATCC8014 on acrylamide-induced oxidative damage in rats

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Abstract
Acrylamide (AA), which is mainly found in fried foods, causes neurotoxicity, genetic toxicity, carcinogenic effects, and DNA damage. This study confirms that a strain of lactic acid bacteria (Lactobacillus plantarum ATCC8014) could alleviate the toxicity of rats by inhibiting the AA-induced oxidative damage. Forty-eight adult male SD rats were randomly divided into eight groups: control group, AA group (40 mg/kg), three different doses (1 × 10⁷ CFU/ml, 1 × 10⁸ CFU/ml, 1 × 10⁹ CFU/ml of Lactobacillus plantarum ATCC8014) of prevention groups and therapeutic groups, respectively. At the end of three-week experiment, AA treatment produced a significant reduction in the rate of weight gain along with the symptoms of hind limb splay and ataxia. Histological examinations revealed various degrees of injury in five tissues. Levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in group AA rats were significantly decreased, but the level of lipid peroxidation (LPO) was significantly increased (p < 0.05). Both prevention and therapeutic groups with 1 × 10⁹ CFU/ml of Lactobacillus plantarum ATCC8014 could effectively reduce the injury of AA to the body. However, reductions in both groups were not statistically significant.

Keywords: Acrylamide, Lactobacillus plantarum, Toxicity in vivo, Adsorption function, Antioxidant

Introduction
Acrylamide (AA) is a water-soluble vinyl monomer that is widely present in fried, baked, and roasted foods, such as French fries, breakfast cereals, and roasted coffee [1, 2]. In 2010 AA was classified as a Category 2 carcinogen and a Category 2 mutagen by the European Commission, as well as a substance of “very high concern” by the European Chemical Agency due to its neurotoxicity, genotoxicity, reproductive toxicity, and possible carcinogenicity [3]. Previous studies have mainly focused on the reduction of AA contents in foods by modifying the processing conditions [4, 5]. However, the presence of AA in food remains unavoidable. Due to its high water solubility and low molecular weight, it can easily cross biological membranes, be absorbed in the gastrointestinal tract, and distributed throughout the body [6]. Several studies have shown that AA exposure could cause dyskinesia [7], growth retardation [8], energy metabolism disorders [9, 10], and genetic damage to germ cells in animals [11]. These symptoms were caused by exposure to AA, which can lead to imbalance in oxidant and antioxidant levels in vivo and to oxidative stress resulting from excessive production of reactive oxygen species (ROS) [12, 13]. Therefore, it is necessary to look for the strategies to reduce the toxicity in vivo by inhibiting the AA-induced oxidative damage.

In recent years, various biotechnology approaches have been used in order to cut down the contents and bioavailabilities of toxic and harmful substances [14, 15]. Lactic acid bacteria (LAB) strains, regarded as safe and...
environmentally-friendly substances, have been shown to have a slight detoxification effect, which has caught people's attention [16]. Several studies have shown that LAB strains have the ability to bind some carcinogens, including patulin, ochratoxin A, fumonisin B₁ and aflatoxins (M₁, M₂, and B₁) in vitro [1, 16–19], and LAB strains could also inhibit toxicity by adsorbing aflatoxin and Cadmium in vivo [20, 21]. However, current research on LAB strains adsorbing AA is limited to in vitro experiments, and there is no data to confirm that it can alleviate AA toxicity in vivo. Studies have shown that peptidoglycan (PGN) on the cell walls of LAB strains is the main site for binding the toxicants. Differences in the composition of PGN and the cross-linking mode of PGN-linked peptides of different bacterial species affect the adsorption capacity of the LAB strains [22–24]. In our previous study, we screened five strains of LAB to explore the stability and mechanism of AA adsorption, and confirmed that *Lactobacillus plantarum* ATCC8014 (*L. plantarum* ATCC8014) has the best adsorption rate of 93.16% and optimal adsorption stability. In addition, the C=O, C–O, and N–H groups in the cell wall are the primary functional groups for *L. plantarum* ATCC8014 to adsorb AA, and the adsorption capacity is related to cell wall roughness [25]. Moreover, *Lactobacillus plantarum* (*L. plantarum*) also has a well-known antioxidant function [26, 27]. We hypothesized that it could have the effect of alleviating the toxicity of AA in vivo. Therefore, the aim of this study was to explore the protective effect of *L. plantarum* ATCC8014 on the AA-induced toxicity in rats and its possible mechanism.

**Materials and methods**

**Material and kits**

AA (99% purity) was purchased from Beijing Boaotuo Co., Ltd (Beijing, China). Bicinchoninic acid (BCA) protein assay, superoxide dismutase (SOD) assay, catalase (CAT) assay, glutathione (GSH) assay, and lipid peroxidation (LPO) assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). AA was dissolved in physiological saline before administration to rats. All other chemicals and reagents employed in this work were of analytical grade and acquired from commercial sources.

**Preparation of AA solution**

The purchased AA was stored in a dry and ventilated place. In the first 5 days of the experiment, 0.8 g of AA was weighed and dissolved in 100 ml of water before being administered to the rats every day. After weighing the rats’ weight on the 6th, 11th and 16th day of the experiment, we adjust the amount of AA weighing according to the average value of rats’ weight in each group, so as to ensure that the intragastric dose of rats is 40 mg/kg/day.

**Bacterial strains and culture**

*Lactobacillus plantarum* ATCC8014 was provided by College of Food Science, Northeast Agricultural University (Harbin, China). The strain was activated in MRS broth. Two sub-cultures were performed to determine the opportune cell concentrations before each experiment. Then, 4% of the LAB strains inocula was added to the MRS broth for 24 h at 37 °C. Bacterial cells were collected by centrifugation (4 °C, 8000 r/min, 10 min) and then washed twice 10 g/L sterilized peptone aqueous solution. After washing, 1 g of bacterial cells were added to the protective agent prepared with 12% defatted milk powder, 2% sucrose and 2% trehalose in 10 mL and mixed in ice water. The pH of bacterial suspension was adjusted to 7.0 with sterile sodium hydroxide. The amount of LAB strains was determined by plate count. Bacterial suspension was freeze-dried to make active powder and stored in the refrigerator at −20 °C. Before gavage, the concentration of LAB strains bacterial suspension was adjusted to 1 × 10⁹ CFU/ml, 1 × 10⁸ CFU/ml and 1 × 10⁷ CFU/ml, respectively.

**Experimental design**

Healthy male SD rats weighing 200±20 g were purchased from the Laboratory Animal Department of Harbin Medical University (animal license number: SCXK 2013-001). The experimental protocol was approved by the Northeast Agricultural University Ethical Committee (China). Care and handling of the animals were in accordance with the national animal research guidelines (Approved by the State Council on October 31, 1988, and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988). Rats were housed in a well-ventilated room with controlled temperature (20–24 °C) and humidity (40–50%) and a 12 h light/dark cycle. Rats were allowed to adapt to their surroundings for 7 days prior to initiating experiments. Rats were given food and water ad libitum throughout the course of the study.

The freeze-dried bacterial powder was dissolved in distilled water and orally administered to each rat for 21 days. *L. plantarum* ATCC8014 was administered 1 h before or after AA exposure as preventive groups and therapeutic groups, respectively.

A total of 48 healthy adult male rats were randomly divided into eight groups:

- **GroupI:** Control group (n = 6), treated orally with physiological saline.
- **GroupII:** AA group (n = 6), administered 40 mg/kg/day AA by gastric gavage.

Group III: Low dose prevention group (n = 6), 1 h before AA administration (40 mg/kg/day), 1 × 10^7 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

Group IV: Medium dose prevention group (n = 6), 1 h before AA administration (40 mg/kg/day), 1 × 10^8 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

Group V: High dose prevention group (n = 6), 1 h after AA administration (40 mg/kg/day), 1 × 10^9 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

Group VI: Low dose therapeutic group (n = 6), 1 h after AA administration (40 mg/kg/day), 1 × 10^7 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

Group VII: Medium dose therapeutic group (n = 6), 1 h after AA administration (40 mg/kg/day), 1 × 10^8 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

Group VIII: High dose therapeutic group (n = 6), 1 h after AA administration (40 mg/kg/day), 1 × 10^9 CFU/ml *L. plantarum* ATCC8014 (1 ml) was given by gastric gavage.

During the 21 days of intervention, body weight, gait scores and hind limb splay were measured every 5 days.

**Behavioral analyses**

**Gait scores test**

Gait scores were measured every 5 days. To observe gait abnormalities, rats were placed in a clear box and were observed for 3 min, and a gait score was assigned from 1 to 4 according to the criteria of numerical gait score, i.e., 1 (unaffected) = a normal gait; 2 (slightly affected) = a slightly abnormal gait (slight ataxia, hopping gait and foot splay); 3 (moderately affected) = moderately abnormal gait (obvious ataxia and foot splay with limb abduction during ambulation); 4 (severely affected) = severely abnormal gait (inability to support body weight and foot splay).

**Hind limb splay examination**

To assess hind limb splay, the hind feet of rats were inked and were held in a horizontal position 32 cm above white paper. Rats were then dropped onto the paper and the distance between the center of the right and left heels was measured. The hind limb heel prints were recorded three times for each rat. The mean values were then calculated for individual rats.

**Collection of tissue samples**

24 h after the final treatment, after ether anesthesia, animals were sacrificed of cervical dislocation and their tissues (hippocampus, cerebellum, liver, small intestine, kidney) were collected. One part of each tissue was fixed in 4% paraformaldehyde for histopathological observation; the other part was stored at -80 °C until oxidative stress indicators were detected.

**Determination of oxidative stress parameters**

After washing hair and blood stains in sterile saline, we weighed 0.1 g hippocampus, cerebellum, liver, ileum part of small intestine and kidney, homogenized them in saline to obtain 10% homogenate (w/v). The supernatant was obtained after centrifugation (2500 r/min, for 10 min). Next, the activities of SOD, CAT, GSH, and the level of LPO were determined according to the kit instructions.

**Histopathological analysis**

Hippocampus, cerebellum, liver, small intestine, kidney tissues of dissected rats were collected and fixed in 4% paraformaldehyde solution. Then, these tissues analysed based on histological tissue follow-up procedures and embedded in paraffin blocks. After the cutting of 5 μm thick paraffin sections, hematoxylin–eosin staining was performed. Then, the histological slides were examined by light microscopy.

**Statistical analysis**

Analysis of data was performed using the computer program SPSS 23.0 for Windows. The data were analyzed by ANOVA and post hoc analysis of group differences was performed by the LSD test. Each experiment was repeated three times and the results were given as the mean ± standard deviation. The statistically significance was considered as p < 0.05.

**Results**

**Effects on body weight**

As shown in Fig. 1, there was no significant difference in the starting body weight among the eight groups of rats (p > 0.05). Body weight increased in each group of rats over the 21-day course of the study, with the weight gain of control rats increasing by 61.57%. Rats in the AA group grew slowly, with a body weight gain rate of 15.99%. From the sixth day on, the body weight in the AA group was significantly higher than the weight gain rate of 61.57%. From the sixth day on, the body weight in the AA group was significantly lower than that of the control group (p < 0.05). Compared to the control group, the weight gain of rats treated with a high dose of *L. plantarum* ATCC8014 (1 × 10^9 CFU/ml) was the greatest significantly higher than the weight gain of the rats in the middle and low dose groups.

**Effects on hind limb splay**

As shown in Fig. 2, at the beginning of AA exposure, rats exhibited a normal degree of hind limb splay. Except for the control group, the hind limb splay in
each group continued to increase with ongoing exposure. After 21 days, the hind limb splay in the AA group increased from 5.67 ± 1.93 cm to 16.58 ± 1.30 cm. The hind limb splay of rats in high dose groups were significantly shorter (p < 0.05) than that of rats in the AA group and the low dose groups, and there was no significant difference between the high dose group and the control group (p > 0.05). The hind limb splay in low dose groups were was close to that in the AA group. In this study, there was no significant difference in hind limb splay between the therapeutic group and the prevention group treated with the same dose of *L. plantarum* ATCC8014 (p > 0.05). (Additional file 1 shows the appearance of each group of rats on the last day of the experiment).

### Effects on gait change

As shown in Fig. 3, 21 days of exposure to AA can induce progressive gait abnormalities in rats, characterized by an unsteady walking pattern with abduction and external rotation of the hind limbs, increased heel splay and decreased locomotor activity. In the AA group, gait scores increased from an initial 1.00 ± 0.00 (normal gait) to 4.00 ± 0.00 by the end of the 21-day exposure period. The treatment of *L. plantarum* ATCC8014 with low, medium and high dose had certain protective effects on gait abnormality in rats. The high dose group had the best results in alleviating abnormal gait. In addition, there was no significant difference on gait change between the therapeutic group and the prevention group treated with the same dose of *L. plantarum* ATCC8014 (p > 0.05).

### Effects on oxidative stress parameters

Enzyme activities of SOD and CAT, and levels of LPO and GSH in tissue samples from the hippocampus, cerebellum, liver, small intestine, and kidneys are provided.
in Table 1. As expected, AA significantly reduced enzyme activities of SOD and CAT, as well as the level of GSH, and it induced an obvious increase in LPO levels, as evident in five tissue samples of the AA rats compared with those of the control rats (p < 0.05). Both in the therapeutic groups and the prevention groups, the rats treated with L. plantarum ATCC8014 had an increase in SOD, CAT activity, and GSH content, while a decrease in the LPO content. Among these, the above parameters were significantly changed in rats treated with high doses of L. plantarum ATCC8014 compared to those of rats in the AA group (p < 0.05).

Effects on histopathology

The hippocampus of the rats in the control group had a clear neuron structure and a certain number of glial cells. Compared with the control group, the hippocampus of the AA group showed degenerative changes, including pale staining or dissolution of nucleus of nerve cells, blurred neural cell structure, and increased number of glial cells showing diffuse proliferation. In the low dose (1 × 10⁷ CFU/ml L. plantarum ATCC8014) therapeutic
group and the prevention group, there was a slight sign of recovery in the hippocampus of the rats, but damage to the nerve cell structure and glial cells still existed. There was no obvious pathological change in the high dose group, and most neurons and glia were normal (Fig. 4).

In the control group, the structure of nerve cells in the cerebellum was clear, granular layer cells were abundant, and Purkinje cells were arranged neatly. The Purkinje cells in the AA group were smaller than those in the control group, showing obvious karyolysis, and the number of granular layer cells was significantly reduced. The protective effect of low dose *L. plantarum* ATCC8014 on AA-induced toxicity was weak but still better than that of the AA group. It showed that the number of Purkinje cells decreased, some cells dissolved, and the structure of cells was blurred. The protective effect of the high dose group was the strongest and without obvious pathological changes. There was no significant difference between the therapeutic group and the prevention group at the same dose (Fig. 5).

Fig. 4 Histopathological sections of hippocampus (H&E ×400). Note: The control group showed evenly stained nerve cells, and nuclei were centrally located with clear nucleoli. The AA group and two low dose groups showed obvious nucleolysis and blurred cell structure, suggesting cell necrosis (black arrow) and increased glial cells. Nerve cell damage was not obvious in two high dose groups.
Liver sections of the rats in the control group had normal histological structure. Congestion in the central vein, sinusoidal, and vascular structures in the portal region were observed in the AA group liver sections. No striate arrangement of hepatocytes was found. In the low dose groups, there was no obvious strand arrangement of hepatocytes, and some nucleolysis, nuclear concentration, and a small amount of lymphocyte infiltration were observed. Compared with the low dose groups, the high dose groups improved these symptoms significantly (Fig. 6).

Mucus and the small intestinal villi of the rats in the control group were arranged regularly. In the AA group, there was an increase in necrosis and exfoliation of mucosal epithelial cells, intestinal villus rupture, vascular congestion, and lymphocyte lamina propria. The epithelial cells of intestinal mucosa of rats in low dose groups were exfoliated, but there was no obvious pathological change in the high dose groups. The protective
The effect of low dose and high dose prevention groups on AA-induced injury was slightly better than that of the therapeutic groups (Fig. 7).

Figure 8 shows normal histological structure of glomeruli and surrounding tubules in the kidneys of control rats. The kidney tissue structure of rats treated with AA showed glomerular atrophy, the tubular epithelial cells became smaller, resulting in a thinner tubular wall, and part of the tubular epithelial cells were necrotic and shed. The protective effect of high dose *L. plantarum* ATCC8014 was significantly better than that of the low dose group, and no obvious pathological tissue damage was observed.
Discussion

At present, most research focuses on reducing the production of AA in food by physical and chemical methods. However, current processing technologies cannot allow people a complete avoidance of AA. It is worth noting that there have been few studies on reducing toxicity in vivo after the AA intake. Therefore, we aimed to explore whether _L. plantarum_ ATCC8014 can alleviate the oxidative stress induced by AA in rats. In addition, we set up the preventive groups and the therapeutic groups at the same time, aiming to preliminary explore whether the protective effect of _L. plantarum_ ATCC8014 before or after the administration of AA was different. So as to lay the foundation for further study on _L. plantarum_ ATCC8014 alleviating AA oxidative damage.

A large number of studies have proved that AA can cause severe damage to the antioxidant system of rats. Continuous exposure of AA leads to excessive production of reactive oxygen species (ROS) and exceeds the load of the antioxidant system [12, 13], resulting in

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**Fig. 7** Histopathological sections of small intestine (H&E × 200). The control group showed clear villus structure. AA group and low dose prevention group showed intestinal villous epithelial cell exfoliation (black arrow). Low dose therapeutic group showed intestinal villus rupture. Two high dose groups showed slight intestinal villus rupture.
the collapse of the antioxidant system in rats, which is manifested by a sharp decrease in antioxidant enzyme (SOD, CAT) activity and the level of antioxidant (GSH) [28–30]. At this time, excessive ROS continuously attacked the cells, and the accumulation of lipid peroxides in the cells eventually caused oxidative damage to various tissues of the rats [31, 32].

Many studies have shown that AA intake can cause slow growth or weight loss in rats [33, 34]. According to our results, the body weight growth rate of rats in the high doses of *L. plantarum* ATCC8014 groups was significantly higher than that of the AA group, and lower than that of the control group, indicating that high doses of *L. plantarum* ATCC8014 can effectively alleviate the slow growth induced by AA in rats.

As a neurotoxic substance, AA damages both the central and peripheral nervous systems and causes rats adverse effects, such as ataxia, skeletal muscle weakness, and other abnormal behavior phenomena [33, 35]. Moreover, AA can reduce the levels of antioxidant enzymes

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**Fig. 8** Histopathological sections of kidneys (H&E ×200). The control group showed normal histological structure of the glomeruli and surrounding tubules in the kidney. The AA group and two low dose groups showed glomerular atrophy, and enlarged bowman's space (black arrow). There was no obvious pathological damage in the two high dose groups.
(SOD, CAT) and GSH, and increase the level of LPO in 
rats [36, 37]. Our results showed that no obvious ataxia 
and hind limb valgus were observed in rats fed high doses 
of \textit{L. plantarum} ATCC8014. Histopathological sections 
showed no obvious hippocampal neuron necrosis and 
 Purkinje cell degeneration, and the number of cerebel-
lar granular layer cells was significantly higher than that 
of the AA group, which further confirmed that \textit{L. plan-
tarum} ATCC8014 had a good mitigation effect on AA-
induced nerve injury in rats. \textit{L. plantarum} ATCC8014 
could effectively enhance the activity of antioxidant 
enzymes and the level of GSH, and could enhance the 
antioxidant capacity of nerve tissue, thus preventing 
ROS from attacking cell membrane for a long time and 
reducing the accumulation of lipid peroxides. In addition, 
we hypothesized that \textit{L. plantarum} ATCC8014 might 
improve the transmission of nerve interference signals 
and the interaction between nerve endings [38], and 
enhanced the activity of cholinesterase in the brain. As a 
potential marker, cholinesterase is thought to be involved 
in the regulation of cholinergic transmission, neurite 
growth and cell proliferation during the development of 
the nervous system [39].

When AA is absorbed from the intestine into the 
blood, it rapidly distributes into the liver, kidneys, and 
other tissues. Most of AA is metabolized to mercapturic 
acid in the liver with the help of GST/GSH system, fil-
tered through the kidneys, and excreted from the body 
with urine [40, 41]. Studies have shown that AA can 
damage liver, small intestine, kidneys and other tissues, 
mainly by inhibiting the levels of antioxidant enzymes 
SOD and CAT, reducing the level of GSH, increasing 
the accumulation of lipid peroxides, and ultimately causing 
tissue damage [31, 32, 42]. According to our experimen-
tal results, high dose of \textit{L. plantarum} ATCC8014 could 
effectively improve the GSH level, as well as the SOD and 
CAT activity in the liver, small intestine, and kidneys of 
rats. In addition, it was evident that these three tissues of 
rats in the high dose group were only slightly damaged, 
and the degree of damage was much lighter than that of 
the rats in the AA group. Therefore, we hypothesized that 
\textit{L. plantarum} ATCC8014 could help eliminate the exces-
tive ROS caused by AA, maintain the balance of oxidants 
and antioxidants in rats, and reduce the damage caused 
by oxidative stress in vivo.

\textit{L. plantarum} ATCC8014 could effectively alleviate AA 
-induced injuries in vivo for the following three poten-
tial reasons. First, \textit{L. plantarum} ATCC8014 has a good 
adsorption effect on AA. Serrano-Nino et al. thought that 
carbonyl oxygen of AA might interact with glucose or 
glycerol hydroxyl in phosphoteichoic acid of LAB strains 
to stabilize its binding [15]. In addition, our previous 
studies have confirmed that the adsorption of AA by LAB 
strains is closely related to the properties of PGN and 
the adsorption capacity is related to the roughness of cell 
wall. C=O, C-O and N–H groups in PGN, as the main 
binding sites, can effectively adsorb AA [25]. Therefore, 
we hypothesized that during the experiment part \textit{L. plan-
tarum} ATCC8014 would stay or colonize the intestinal 
tracts of rats and might combine with AA to form stable 
complexes. Research by Gratzi et al. shows that complexes 
formed by \textit{Lactobacillus rhamnosus} GG adsorbing afla-
toxin B1 are more likely to be excreted from the body 
through feces [43], so we hypothesized that \textit{L. plantarum} 
ATCC8014—AA complexes might be excreted from the 
body rather than absorbed by the intestinal tract, reduc-
ing the absorption of AA and further alleviating the tox-
icity of AA in vivo. Second, many probiotics, including \textit{L. plan-
tarum}, protect the intestinal barrier by upregulating 
the expression of tight junction proteins [44, 45], and 
can even reduce the absorption of toxic substances in the 
intestine [46]. Therefore, \textit{L. plantarum} ATCC8014 might 
have a certain protective effect on the intestine—enhanc-
ing the intestinal barrier function and preventing AA 
from entering the body. Finally, some studies have shown 
that \textit{L. plantarum} also has well-known antioxidant func-
tions. In vitro, \textit{L. plantarum} has an effective free rad-
ical scavenging ability and reducing ability [47, 48], and 
can enhance the antioxidant enzyme activity of Caco-2 
cells [49]. In vivo, \textit{L. plantarum} not only enhances the 
antioxidant status of the kidney [50], liver [47, 51], and 
heart [52], but also enhances the activity of antioxidant 
enzymes in the plasma of rats, and enhances the overall 
antioxidant level of rats [50]. Although the antioxidant 
activity of lactic acid bacteria has been confirmed by 
in vivo and in vitro tests, its specific antioxidant mecha-
nism is not clear enough and needs further investigation. 
Some evidence suggests that \textit{L. plantarum} may produce 
antioxidant substances, including polysaccharides, pro-
teins, vitamins and glutathione [20], and the antioxidant 
enzymes contained in its own antioxidant system [53, 
54]. The extensively studied exopolysaccharides have a 
strong ability to scavenge free radicals [55], and they can 
reduce the accumulation of lipid peroxides by stimulat-
ing the activity of antioxidant enzymes in mice, and ultima-
tely reduce the oxidative stress in mice [56]. Therefore, 
\textit{L. plantarum} ATCC8014 might produce polysaccha-
drides, proteins and other substances, which can stimu-
late the antioxidant system of rats to enhance their own 
antioxidant capacity. At the same time, the antioxidant 
enzymes produced by the bacteria themselves also par-
ticipate in the antioxidant process in rats. These antioxi-
dant substances penetrate the intestinal epithelium, enter 
the bloodstream, and are dispersed throughout the body 
while targeting organs and tissues [57], which effectively 
reduces AA-induced toxicity in rats.
According to our experimental results, the prevention group had a better alleviating effect than the therapeutic group. After our analysis, we concluded that the reasons for this might be that in the prevention group, L. plantarum ATCC8014 entered the intestine before AA stayed or colonized. These bacteria might have reduced absorption of AA by enhancing the intestinal barrier function. In addition, part of AA might be excreted by binding to bacteria forming complexes, thus further reducing the absorption of AA.

Supplementary information

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Authors’ contributions

The authors thank all of the study participants. All the participants conceived and designed the study; ZSJ and ZXD conducted experimentation on animals; JY, LYY and XXH guided all experiments; ZXD, FWX and LQB carried out all statistical analysis; ZSJ and SML completed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflicts of interest.

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