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Probiotic microorganisms affect the reproductive and nervous systems of male rats treated with acrylamide

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ABSTRACT

Objective: To evaluate the protective effects of probiotic microorganisms on the reproductive and nervous systems of male rats treated with acrylamide.

Methods: Thirty-two rats were randomly divided into 4 groups and received normal saline through gavage (control), acrylamide 20 mg/kg body weight, acrylamide plus probiotic microorganisms (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Streptococcus thermophilus* and fructooligosaccharides, all mixed in sachets) 20 or 200 mg/kg body weight, respectively. After 30 days, the testis, prostate, seminal vesicle and cerebellum were removed, fixed and stained with hematoxylin-eosin (H & E). The Johnsen score was used to classify spermatogenesis. Cavalieri's principle method was used to evaluate the total volume (in mm³) of the testes. The number of each intratubular cell type as well as intertubular Leydig cells in whole samples was measured using the physical dissector counting techniques. Stereological analysis and the grids were used to determine the volume of cerebellar layers as well as the Purkinje cell number.

Results: The testis weight decreased significantly in the acrylamide-treated group compared to the other groups ($P<0.001$). The number of spermatogonia, spermatocytes, spermatids and Leydig cells in the acrylamide-treated group were significantly less compared to the control group ($P<0.05$), while they were increased significantly in the acrylamide+200 mg/kg probiotic group ($P<0.05$; $P<0.01$). The mean Johnsen score in the acrylamide-treated group was lower than in the control group ($P<0.001$). Acrylamide-induced changes including congestion, vacuolization in the secretory epithelial cells, and epithelial rupture were observed in the prostate and seminal vesicle. The volumes of cerebellar layers were decreased in the acrylamide group compared to the control group while recovered in both probiotic treated groups.

Conclusions: Probiotic microorganisms alleviate acrylamide-induced toxicities against the reproductive and cerebellar tissues in rats.

KEYWORDS: Acrylamide; Cerebellum; Microorganisms; Probiotics; Prostate; Rats; Testis

Significance

Probiotics can help prevent tissue damage by absorbing acrylamide compounds in the body. The findings demonstrated that exposure to acrylamide could decrease testis weight, change its histomorphology, and damage the prostate and seminal vesicle gland. Probiotics might protect against these impacts *via* antioxidant and free radical-scavenging properties. Moreover, probiotics can reduce the neurotoxic effects of acrylamide on the cerebellum. More research is required to fully understand the benefits of probiotics on animal reproductive and nervous systems.

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1. Introduction

Acrylamide is a colourless, odourless, low-weight compound with a molecular formula (C_3H_5NO) that is formed in foods containing high-temperature-cooked carbohydrates during the Millard reaction[1,2]. Genotoxicity and carcinogenicity effects of acrylamide are *via* its glycidamide metabolite, which has more mutagenic potential compared to acrylamide. Glycidamide can make bonds with $-SH$, $-OH$ and $-NH_2$ groups in various molecules, inducing mutations in cellular DNA and alterations in the physiological functions of enzymes[3,4]. In addition, it is used in research laboratories performing gel electrophoresis. The discovery of acrylamide in food in 2002 as a potentially toxic substance attracted researchers' interest[5]. Acrylamide has low molecular weight and solubility in water; therefore, it could cross the cell membrane and damage DNA by oxidation. As a result, cytotoxic effects on various tissues occur, for instance, the reproductive tract, nervous system, intestine, smooth and skeletal muscles, liver, kidney and lung. There is research on acrylamide effects in numerous animal models[6,7]. For example, Ivanski *et al* reported toxic effects in the reproductive system of male rats treated with acrylamide, including functional impairment and tissue damage[8].

Also, acrylamide is a highly potent neurotoxin with a potential effect on both central nervous system and peripheral nervous system in humans and animals[9]. Acrylamide neurotoxicity may be associated with axonal swelling in the long myelinated fibres and can damage nerve terminals as well as Purkinje neurons[10].

Probiotics are living microorganisms that, if taken enough, have beneficial effects on the host's health[11]. They modulate functions of the gastrointestinal tract and immune system, reduce food allergies and prevent cancer *via* their antioxidant effects[12]. Probiotics are beneficial in the regulation of intestinal microbiota, modulation of bacterial metabolism and enzymes, improving mucosal barrier function, production of antimicrobial peptides, and disruption of quorum sensing signalling. They inhibit pro-inflammatory molecules and secrete anti-cancer metabolites[13]. Probiotic microorganisms reduce or may eliminate carcinogenic compounds *in vivo* and prevent tissue damage through their ability to physically absorb carcinogenic compounds such as acrylamide and to prevent acrylamide formation by producing the enzyme *L*-asparaginase[14]. Cuevas-González *et al* showed the preventive property of intracellular content of some probiotic bacteria against acrylamide-induced human erythrocyte injury[15]. Recent studies showed that probiotics have a positive effect on neuropsychiatric and behavioural pathology[16]. In addition, prebiotics have been shown to improve microbiota and may have beneficial effects on the brain. However, the potential modulation of neuronal responses in brain development by prebiotics that may be due to maternal exposure to neurotoxins has been less studied[17]. Although there are ways to reduce acrylamide in food, they are not practical because of the need for expensive equipment or high energy and their adverse effects on food quality. As a result, the use

of probiotics has received special attention[18,19].

Due to the importance of this issue, this study was planned to evaluate the impact of probiotic microorganisms on the reproductive and nervous systems of male rats treated with acrylamide.

2. Materials and methods

2.1. Chemicals

The probiotic microorganisms (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Streptococcus thermophilus* and fructooligosaccharides, all mixed in sachets) were obtained from a pharmaceutical company (Zist Takhmir Co., Tehran, Iran). The hematoxylin-eosin (H & E) stain and acrylamide were obtained from Sigma, USA.

2.2. Experimental design

Thirty-two adult male albino Wistar rats (3 months old and weighing 350-450 g) were provided from the animal laboratory of the Yazd Reproductive Sciences Institute at Shahid Sadoughi University of Medical Sciences in Yazd, Iran. All rats were kept under standard laboratory conditions (free access to water and food, 12/12 h light/dark cycle, temperature 18 °C-23 °C, and humidity 55%), and were randomly allocated into four groups ($n=8$ per group). Group 1 received normal saline and served as the control group; group 2 received only acrylamide 20 mg/kg body weight; groups 3 and 4 were given a mixture of probiotic microorganisms and prebiotic fructo-oligosaccharides (FOS) at doses of 20 and 200 mg/kg by gavage, respectively, in addition to 20 mg/kg acrylamide. The study lasted for 30 days during which rats were administered *via* gavage every other day, and their weight was measured weekly. If any of the rats had a disease or infection, or died during the intervention period, they were excluded from the study.

2.3. Tissue preparation and histological analysis of testis, prostate and seminal vesicle

After 30 days, the perfusion of the heart was done with formalin for fixation of the tissues. Then, the left testicle, prostate and seminal vesicle were removed. Samples were weighed by digital scales and fixed in the Bouin solution. The mean of testis weights in different groups were weighted, too.

Then, paraffin blocks were prepared. Serial sections were made by microtome at 5-6 μm thickness before staining with H & E. The stained sections were studied under light microscopy with magnifications 40 $\times$, 100 $\times$ and 400 $\times$ (Olympus, Japan) to detect changes in spermatogenesis and histopathology. The Johnsen score (Table 1) was used to classify the spermatogenesis. This method

Table 1. Modified Johnsen score.

Score	Description
10	Complete spermatogenesis and perfect tubule
9	Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
8	Less than five spermatozoa per tubule, few late spermatids
7	No spermatozoa, no late spermatids, many early spermatids
6	Few early spermatids, arrest of spermatogenesis at the spermatid stage, disturbance of spermatid differentiation
5	No spermatids, many spermatocytes
4	Few spermatocytes, arrest of spermatogenesis at the primary spermatocyte stage
3	Spermatogonia only
2	No germ cells, Sertoli cells only
1	No germ cell or Sertoli cells, tubular sclerosis

allocates a score of 1-10 to each tubular cross-section examined. In each testis, the epithelia of at least 60-70 tubules were evaluated to calculate the mean Johnsen score for each rat.

2.4. Stereological assessment of testis

2.4.1. The volume of testis

Cavalieri's principle method was used to evaluate the total volume (in mm³) of the testes in the rats. According to the routine histological procedures, the paraffin blocks of testis specimens were sectioned at 5 µm by the microtome through systematic random sampling. Serial sections, with a particular thickness and a specific distance, were made consecutively from each whole testis. A point counting grid with a fixed distance of 100 µm between points was placed randomly on the testis specimen images and the number of points that hit the specimen surface area was counted. Conventionally, points from the sample that hit the top and right were counted.

Then, the following formula was used to calculate the total volume of the testis:

$$V = t \times \frac{a}{p} \times \sum \frac{P}{M^2}$$

V: the total volume of the testis; t: the thickness of the section; a/p: the area of one point (1000 µm); P: count of points enumerated in the specified area; M: magnification[20].

Since the amount of shrinkage did not differ in groups, the shrinkage was not corrected in this experimental study.

2.4.2. The number of Sertoli cells, spermatogonia, spermatocytes, spermatids and Leydig cells

The number of each intratubular cell type as well as intertubular Leydig cells in whole samples was measured using the physical dissector counting techniques. A dissector is a three-dimensional probe used to enumerate the number of objects (cells), with the possibility that it is not affected by the size of the objects, their shape or spatial position. This 3-dimensional dissector probe has two parallel planes used to count the number of cells. Two serial consecutive sections, selected randomly, are evaluated and compared. The first selected section, called the reference section, is used for comparison with the adjacent section called the look-up section. These used sections were 30 µ apart as a rule of physical

dissector. To be measured, the section pairs must be about 30%–40% of the mean projected height of the object of interest (here, cell nuclei) distant from each other. During this procedure, twenty serial dissector pairs from each biopsy were studied. Those cells present in the look-up section which did not touch forbidden lines were counted.

The following formula was used to estimate the mean numerical density of the cell nuclei:

$$NV = \sum \frac{Q}{t} \times a(p) \times h \times p$$

Q: the number of the cell nuclei counted in the reference section; t: the average thickness of section (5 µm); a (p): the area of the unbiased counting frame; h: the distance between sections; p: the number of frames associated points hitting the sample.

The total count of cells in a whole testis was estimated by the following equation:

$$N_{total} = N_v \times V_{total}$$

N_v: the numerical density of cells; N_{total}: the total number of cells in the entire testis calculated using the testis volume results determined by the Cavalieri method[21].

2.5. Histopathologic changes in the prostate and seminal vesicle

Each prostate and seminal vesicle was cut at sections 5-6 µm thick and stained with H & E. The stained sections were studied under both low-power and high-power fields of light microscopy with magnifications 40×, 100× and 400× (Olympus, Japan).

2.6. Histology and stereology assessment of the cerebellum

To evaluate the histology of the cerebellum, the entire cerebellum was dissected before insertion into formalin at room temperature for 48 h. For light microscopic study, sections (5 µm thick) were stained with the H & E method. To determine the volume of cerebellar layers as well as the Purkinje cell number, stereological analysis and the grids were used. Using the Cavalieri's Principle method, the volume of the cerebellum was estimated.

2.7. Statistical analysis

GraphPad Prism version 8.00 (GraphPad Software, San Diego, CA, USA) was used. Statistical tests included parametric one-way analysis of variance (ANOVA) and *t*-pair tests were used for comparison among different groups. Data in normal distribution were expressed as mean±standard deviation (mean±SD). *P*<0.05 was considered statistically significant.

2.8. Ethics statement

All of the experiments were performed under respective national guidelines and animal use which was approved by the institutional Ethics Committee in Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Ethics Code No: IR.SSU.REC.1397.077), including the ARRIVE guidelines.

3. Results

3.1. Johnsen score for spermatogenesis

The mean Johnsen scores for grading of spermatogenesis are shown in Figure 1A. Its mean was quite significantly lower in the acrylamide-treated group compared to the control group ($P<0.001$) and (in a dose-dependent manner) higher in probiotic groups compared to the acrylamide group ($P<0.001$).

3.2. The mean total volume of testis of each group

No significant difference in the total volume of testis in the acrylamide group was observed as compared to the control group. The volume of testis in the acrylamide+20 mg/kg probiotic and acrylamide+200 mg/kg probiotic groups showed a non-significant increase as compared with the acrylamide group. The groups of acrylamide+20 mg/kg probiotic and acrylamide+200 mg/kg probiotic were not significantly different when compared with the control group (Figure 1B).

3.3. Estimation of the number of various cell types in different groups

The total number of spermatogonial cells and spermatocytes in the acrylamide group was significantly lower compared to the control group ($P<0.05$). While the acrylamide + 200 mg/kg probiotic group had a significant increase in spermatogonial cells and spermatocytes compared to the acrylamide group ($P<0.01$) and the acrylamide +20 mg/kg probiotic group ($P<0.05$). Regarding the total count of spermatids in the acrylamide group, it was significantly less than the control group ($P<0.01$). The number of spermatid cells was significantly increased in the acrylamide + 20 mg/kg probiotic group ($P<0.05$) and acrylamide + 200 mg/kg probiotic group ($P<0.01$) compared to the acrylamide group. In addition, the acrylamide + 200 mg/kg probiotic group had higher spermatid cells than the acrylamide + 20 mg/kg probiotic group ($P<0.05$).

The Sertoli cell count in the acrylamide group was lower when

compared with the control group, but the difference was not significant. The number of Sertoli cells in the acrylamide + 20 mg/kg probiotic and acrylamide + 200 mg/kg probiotic groups was higher compared with the acrylamide-treated group, although the differences were not significant. The number of Leydig cells in the acrylamide group was significantly less than in the control group ($P<0.01$). The acrylamide + 200 mg/kg probiotic group had a significant increase in the Leydig cell number compared to the acrylamide group ($P<0.01$) (Table 2).

3.4. Histopathological findings in testis

The testis of the control group showed normal histological structure. Tubular lumina showed normal spermatogenic cell layers and spermatozoa, with normal Leydig cells in the interstitium (Figure 2A). In the acrylamide-treated group, seminiferous tubules showed degeneration of spermatogenic cells with the formation of giant spermatids. Only a few spermatogonia and Sertoli cells remained. Some detached and pyknotic spermatids were observed, and some epithelial cells were vacuolated. In this group, interstitial oedema also was observed (Figure 2B). Testis of acrylamide plus probiotic-treated groups showed mild degeneration in some seminiferous tubules with interstitial oedema. The results showed that the seminiferous tubules in both experimental groups (especially high-dose probiotics) had regular size with regular cytoarchitecture. An overall dose-dependent recovery was seen in the spermatogenic lineage (Figure 2C and 2D).

3.5. Histological findings in the prostate

In the control group, there were not any histopathological changes. Epithelial and stromal elements in the control group depicted normal structure. It has alveolar and tubular units in addition to loose connective tissue (Figure 3A). Various degrees of epithelial hyperplasia were detected in all of the sections from the acrylamide-treated group. The epithelium lost its integrity in this group. We found congestion in the blood vessels of the connective tissue which could potentially result in tissue destruction. Rupture in glandular epithelia was also noted (Figure 3B). The prostate of acrylamide plus

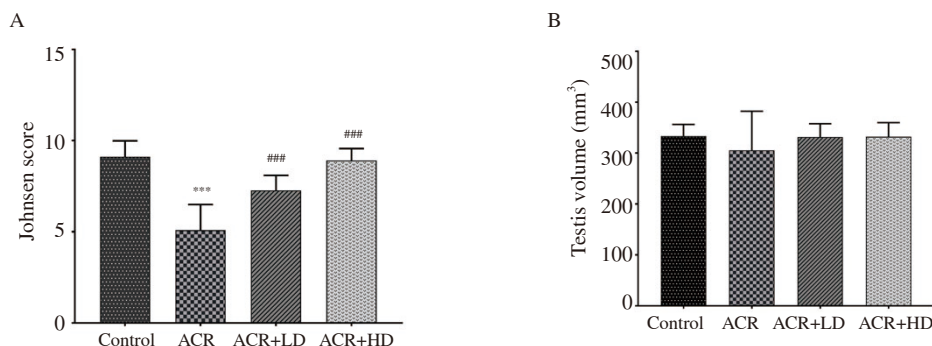


Figure 1. A: Mean Johnsen score of spermatogenesis in different groups. **B:** The mean volume of the testis (mm^3) in different groups. ACR: acrylamide; LD: low dose (20 mg/kg) probiotics; HD: high dose (200 mg/kg) probiotics. *** $P<0.001$: compared to the control group; ### $P<0.001$: compared to the acrylamide (ACR) group.

Table 2. Estimated number of spermatogonia, spermatocyte, spermatid, Sertoli cells and Leydig cells in different groups.

Parameters	Control	Acrylamide	Acrylamide + 20 mg/kg probiotic	Acrylamide + 200 mg/kg probiotic
Spermatogonia number	7.21±0.22	5.00±0.22 ^{a*}	5.15±0.21	7.04±0.21 ^{b**} , c [†]
Spermatocyte number	17.30±0.21	11.39±0.12 ^{a*}	12.45±0.41	16.89±0.32 ^{b**} , c [†]
Spermatid number	9.00±0.14	2.75±0.33 ^{a**}	6.95±0.18 ^{a**} , b [†]	8.21±0.18 ^{b**} , c [†]
Sertoli cell number	2.00±0.12	1.00±0.12	1.14±0.13	1.90±0.07
Leydig cell number	4.64±0.23	2.00±0.01 ^{a**}	3.08±0.52	3.98±0.31 ^{b**}

Data are expressed as mean±SD. a: Compared to the control group, ^{*}P<0.05, ^{**}P<0.01; b: Compared to the acrylamide-treated group, ^{*}P<0.05, ^{**}P<0.01; c: Compared to the acrylamide + 20 mg/kg probiotic group, [†]P<0.05.

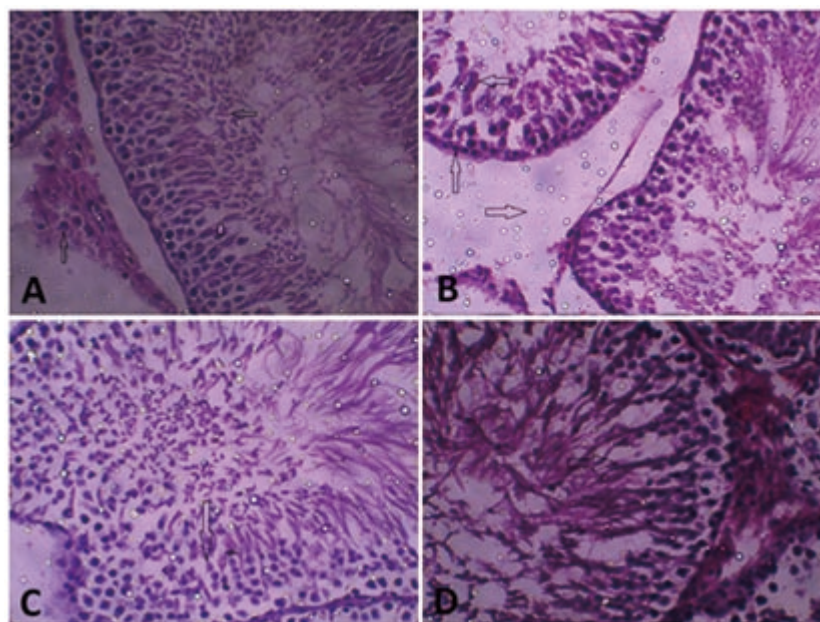


Figure 2. Testis histopathological features of Sertoli cells, spermatogonia, spermatocytes, spermatids and Leydig cells in different groups (400×, H & E staining). The testis of the control group (A) shows normal histological structure. Tubular lumina shows normal spermatogenic cell layers and spermatozoa (left arrow), with normal Leydig cells (up arrow) in the interstitium. In the acrylamide-treated group (B), seminiferous tubules show degeneration of spermatogenic cells with formation of giant spermatids (left arrow). Only few spermatogonia and Sertoli cells are remained (up arrow). Some detached and pyknotic spermatids are observed, and some epithelial cells are vacuolated. In this group, interstitial edema (right arrow) is also observed. Testis of acrylamide plus probiotic treated groups (C, D) show mild degeneration in some seminiferous tubules (down arrow) with interstitial edema. The results show that the seminiferous tubules in both experimental groups (especially high dose probiotic) have regular size with regular cytoarchitecture (right arrow). An overall dose-dependent recovery is seen in the spermatogenic lineage. A, the control group; B, the acrylamide-treated group; C, the acrylamide + low dose probiotics; D, the acrylamide + high dose probiotics.

probiotic-treated groups showed only mild stromal degeneration and rupture in the epithelium (Figure 3C and 3D).

3.6. Histological findings in the seminal vesicle

In the control group, the seminal vesicle gland demonstrated many normal mucosal folds covered by simple or pseudo-stratified columnar epithelium (Figure 4A). In the acrylamide-treated group, the glandular epithelia showed a loss of integrity (Figure 4B). Seminal vesicles in the acrylamide plus probiotic-treated groups showed mild degeneration and rupture in epithelial integrity in a dose-dependent manner (Figure 4C, 4D).

3.7. Histological findings in the cerebellum

Compared with the control group, the volumes of cerebellar layers

were decreased in the acrylamide group while increasing in the acrylamide+200 mg/kg probiotics as well in acrylamide+20 mg/kg probiotic groups in a dose-dependent way. Figures 5 show the status of volumes of the cerebellar layers in the four groups. Figure 6 depicts photomicrographs of cerebellar tissues.

4. Discussion

There are multiple experimental studies denoting the effects of acrylamide on male fertility. In this study, significant reductions were observed in the testes' weight in the acrylamide-treated group compared to the control group which aligns with similar studies. According to Wang *et al*, acrylamide reduces testicular weight and sperm production in male rats[22]. In addition, Sengul *et al* demonstrated that acrylamide induces testicular dysfunction and

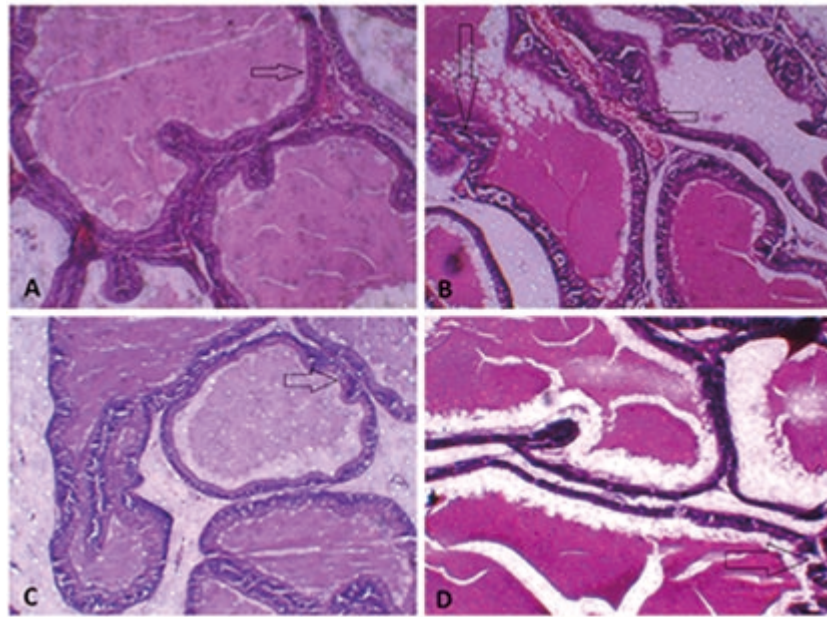


Figure 3. Histopathological changes in prostate in different groups (200 $\times$, H & E staining). In the control group (A), there are not any histopathological changes. Epithelial and stromal elements in the control group depict normal structure (right arrow). It has alveolar and tubular units in addition to a loose connective tissue. In the acrylamide-treated group (B), various degrees of epithelial hyperplasia are detected in all of the sections. The epithelium loses its integrity in this group (down arrow). Congestion in the blood vessels of the connective tissue (left arrow) are found, which could potentially result in tissue destruction. Rupture in glandular epithelia is also noted. Prostate of acrylamide plus probiotic treated groups (C, D) show only mild stromal degeneration and rupture (right arrow) in epithelium.

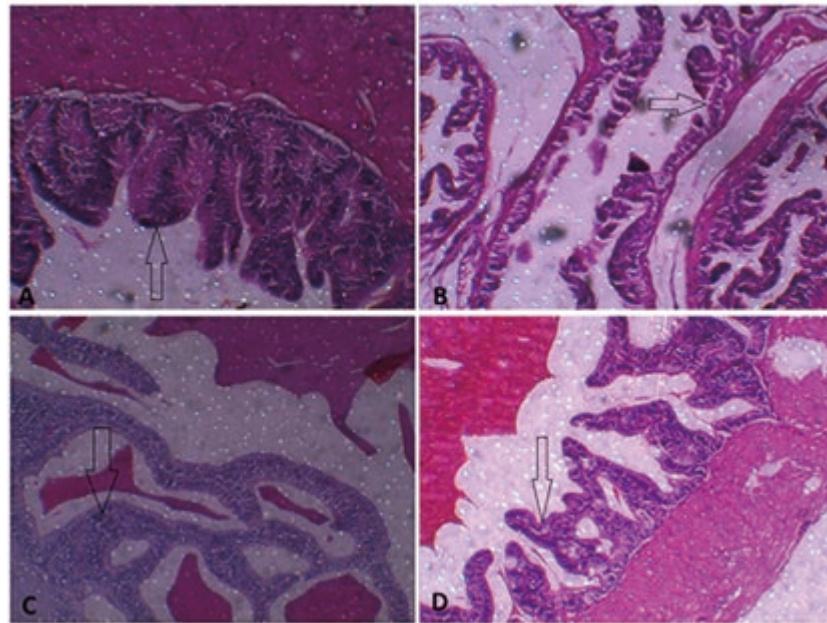


Figure 4. Histopathological changes of seminal vesicle in different groups (200 $\times$, H & E staining). In the control group (A), seminal vesicle gland demonstrates many normal mucosal folds (up arrow) covered by simple or pseudo-stratified columnar epithelium. In the acrylamide-treated group (B), the glandular epithelia show loss of integrity (right arrow). Seminal vesicles in the acrylamide plus probiotic treated groups (C, D) show mild degeneration (down arrows) and rupture in epithelial integrity in a dose dependent manner.

tissue damage in rats[23]. Therefore, the results of these studies were similar to our findings.

The total number of spermatogonia and spermatocytes in the acrylamide group was significantly less than the control and

acrylamide+200 mg/kg probiotic groups. Likewise, the total number of spermatids in the acrylamide group was significantly below the level in the control group, while the number of these cells was more in the acrylamide+200 mg/kg probiotic as well

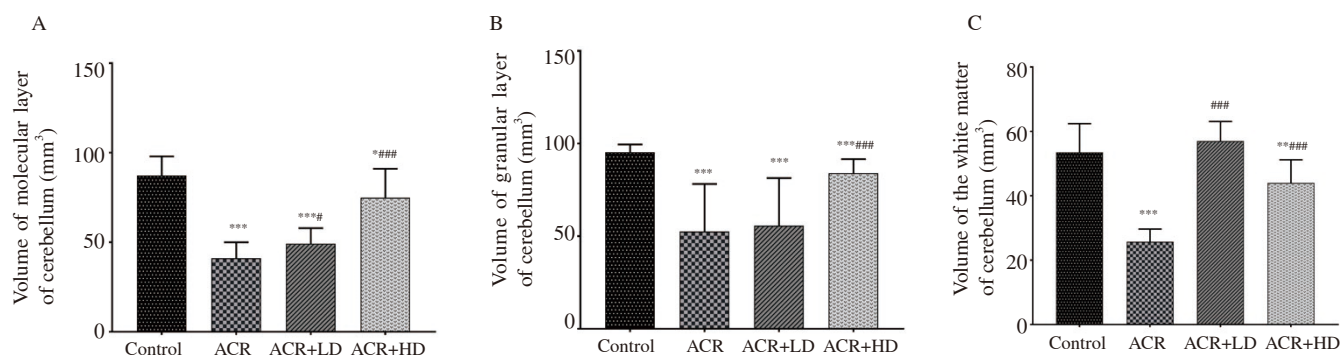


Figure 5. The mean volume of molecular layer (A), granular layer (B) and white matter layer (C) in different groups. * $P<0.05$, ** $P<0.01$, *** $P<0.001$: compared to the control group; # $P<0.05$, ### $P<0.001$: compared to the ACR group. ACR: acrylamide; LD: low dose (20 mg/kg) probiotics; HD: high dose (200 mg/kg) probiotics.

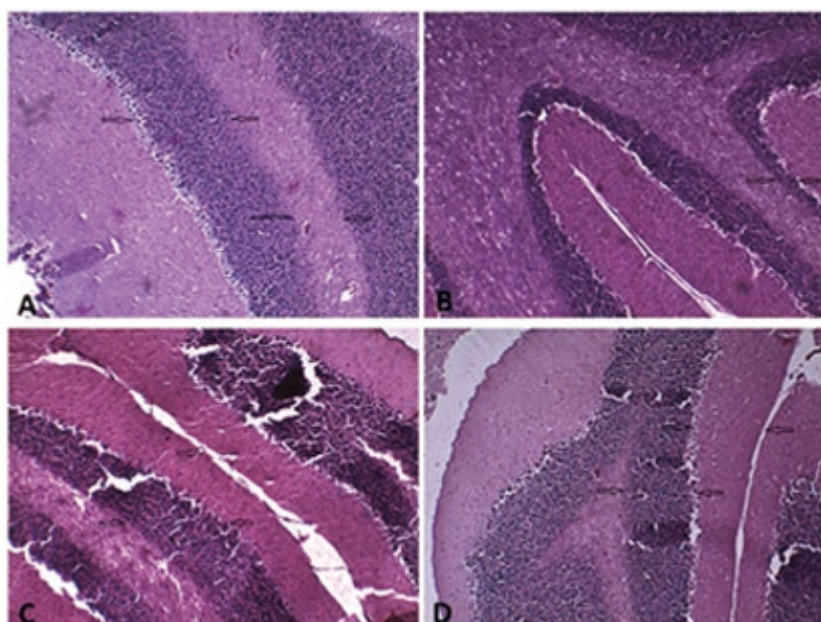


Figure 6. Cerebellar histopathological changes in different groups (100×, H & E staining). A, the control group; B, the acrylamide treated group; C, the acrylamide + low dose probiotics; D, the acrylamide + high dose probiotics. Arrows on both sides of a cerebellar layer point to the thickness of layers, allowing comparison between the 4 groups (being lowest in the group B).

as acrylamide+20 mg/kg probiotic groups in a dose-dependent manner.

The number of Sertoli cells in the acrylamide group turned out to be less when compared with the control group, but not significantly. The number of Leydig cells in the acrylamide group was significantly lower in comparison with the control and acrylamide+200 mg/kg probiotic groups. Previous studies demonstrated that acrylamide reduces the testis volume which could be due to a decreased number of germ cells[22,24]. Acrylamide has been found to induce histopathological changes in male reproductive organs, *e.g.*, vacuolation and multinucleation in addition to apoptosis in the cells of the seminiferous tubules[24,25]. Acrylamide may cause epithelial thickening in seminiferous tubules, which consequently decreases sperm production[26]. The reduced number of intratubular cells in our study could be due to increased apoptosis. Consistent

with our findings, Wang *et al* showed that acrylamide can decrease the number of epididymal spermatozoa and may induce structural lesions in rat testes[22].

Probiotics are live microbial preparations exerting useful effects on health. Antioxidant supplementation may protect and prevent peroxidative damage[27]. To our knowledge, few studies have attributed probiotics to the reproductive system. Kistanova *et al* reported that probiotics could be used for the improvement of reproductive performances[28]. Choi *et al* studied the efficacy of probiotic bacteria in lowering acrylamide levels in food and during *in vitro* digestion. They found that probiotic strains can decrease acrylamide in chemical solutions and two specific food products (biscuits and potato chips)[29]. In addition, acrylamide leads to increased systemic inflammation in rats and increases the level of pro-inflammatory cytokines, and probiotics can moderate this

inflammation[30]. We also demonstrated that the administration of probiotics could potentially mitigate the detrimental impacts of acrylamide.

The current study revealed that the use of probiotics improved the reproductive organs of rats in a dose-dependent manner. We found that probiotics could repair acrylamide-induced damage and reinstate the spermatogenic cell population of the seminiferous tubules to some extent.

Acrylamide is a well-known neurotoxin in both humans and animals. A previous study suggests that both human and animal neurotoxicities are mediated by the degeneration of distal axons of cerebellar Purkinje cells in the peripheral and central nervous system[31]. Dortaj *et al* demonstrated decreased cerebellar weight and also lower number of Purkinje cells in newborns of acrylamide-treated female rats. Acrylamide also decreased the volumes of the granular and molecular layers while increasing the volume of white matter. Acrylamide was able to induce histology-proven changes in the cerebellar cortical layers of rat newborns, which were preventable by vitamin C[32]. In agreement with their study, in the present study, exposure to acrylamide caused a significant fall in volume of the molecular layer, granular layer and average number of cerebellar Purkinje cells, likely the result of damage to nerve terminals in these regions. It also decreased the volume of cerebellar white matter. The use of probiotics in this study reduced the damaging effects of acrylamide.

The study has some limitations. In this study, we can mention the time constraints arising from rats' mortality after over 30 days of acrylamide treatment, along with the escalation of oesophagus and throat damage and infection in rats during the gavage. Further research could involve using more intervention material, studying increasing probiotic doses for the experimental groups, and conducting a comprehensive analysis of blood and inflammatory parameters when collecting blood samples from different groups. Moreover, to enhance the assessment of probiotics' protective effects against carcinogens like acrylamide, it is recommended to investigate more tissues.

In conclusion, exposure to acrylamide may decrease testis weight and change its histomorphology; these effects could be ameliorated by simultaneous administration of probiotic microorganisms. The results also revealed that acrylamide has destructive effects on the prostate and seminal vesicle gland. Probiotics could protect against these effects. Theoretically, this could be explained by its antioxidant and free radical-scavenging activities. The present study suggests that the neurotoxic effects of acrylamide on the cerebellum can be diminished by probiotics; this, too, might be a merit of its antioxidant properties. The mechanisms behind the beneficial actions of probiotics in improving reproductive and cerebellar tissues in animals need further studies.

Conflict of interest statement

The authors have no conflict of interest.

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Authors' contributions

Seyedhossein Hekmatimoghaddam and Seyed Mohammad Seifati contributed to the study's conception and design. Material preparation, animal interventions, data collection and analysis were performed by Seyed Mohammad Seifati, Maryam Yadegari, Fatemeh Zakizadeh and Fateme Zare. The first draft of the manuscript was written by Seyed Mohammad Seifati and Seyedhossein Hekmatimoghaddam. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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