RESEARCH ARTICLE

The ameliorative effect of *Lactobacillus paracasei* BEJ01 against FB1 induced spermatogenesis disturbance, testicular oxidative stress and histopathological damage

Khawla Ezdini\(^a\), Jalila Ben Salah-Abbès\(^a\), Hela Belgacem\(^a\), Bolanle Ojokoh\(^b\), Kamel Chaieb\(^c\) and Samir Abbès\(^a,d\)

\(^a\)Laboratory of Genetic, Biodiversity and Bio-resources Valorisation, University of Monastir, Monastir, Tunisia; \(^b\)Department of Information Systems, Federal University of Technology, Akure, Nigeria; \(^c\)Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; \(^d\)Higher Institute of Biotechnology of Béja, University of Jendouba, Jendouba, Tunisia

ABSTRACT

Fumonisin B1 (FB1) is a possible carcinogenic molecule for humans as classified by the International Agency for Research on Cancer (IARC) in 2B group. In livestock, it is responsible for several mycotoxins and economic losses. *Lactobacillus* strains, inhabitants of a wide range of foodstuffs and the gastrointestinal tract, are generally recognized as safe (GRAS). Thus, the aim of this work was to evaluate the protective effect of *Lactobacillus paracasei* (LP) against FB1-induced reprotoxicities including testicular histopathology, sperm quality disturbance, and testosterone level reduction.

Pubescent mice were divided randomly into four groups and treated for 10 days. Group 1: Control; Group 2: FB1 (100 μg/kg b.w); Group 3: LP (2 x 10^8 CFU/kg b.w); Group 4: LP (2 x 10^9 CFU/kg b.w) and FB1 (100 μg/kg b.w). After the end of the treatment, animals were sacrificed. Plasma, epididymis, and testis were collected for reproductive system studies.

Our results showed that FB1 altered epididymal sperm quality, generated oxidative stress, and induced histological alterations. Interestingly, these deleterious effects have been counteracted by the LP administration in mice.

In conclusion, LP was able to prevent FB1-reproductive system damage in BALB/c mice and could be validated as an anti-caking agent in an animal FB1-contaminated diet.

1. Introduction

Fumonisin B1 (FB1) is one of the prevalent mycotoxins, produced by toxigenic fungi such as *Fusarium verticillioides* and *Fusarium moniliforme*. It is naturally present in a large variety of food and foodstuff with high incidence. In fact, FB1 is the most widely distributed fumonisin mycotoxin in North Africa (74%). In Tunisia, Jedidi et al. (2021) detected high fumonisin levels in wheat (20.83%), barley (40%), and maize (57.14%). These levels exceeded European limits and hence, suggested a risk for Tunisian cereals and consumers. This toxin was linked to human cancers (IARC 2002). Experimentally, FB1 toxicity can be traced to its analogy to sphingoid bases and the inhibition of ceramide synthase, a key enzyme in the biosynthesis of sphingolipids (Dellaflora et al. 2018).

Indeed, a large body of evidence has demonstrated that FB1 leads to several in vivo and in vitro biological alterations. In fact, Cao et al. (2020) reported that FB1 affected the intestines, the first defense line of microorganisms against toxins, and caused homeostasis imbalance via the disturbance of Cyto P450 activity. Other studies demonstrated that liver and kidney, specific hosts for FB1 accumulation, could also be damaged (Rumora et al. 2007; Demirel et al. 2015; Hou et al. 2021). Moreover, FB1 was found to generate oxidative stress markers (Baldissera et al. 2020), neurochemistry toxicity (Gbore 2010), seizures, as well as hyperexcitability (Poersch et al. 2015) in the brain. Several studies reported also that FB1 induced immunotoxicity (Abbès et al. 2016), apoptosis (Kim et al. 2018; Zhang et al. 2018), and genotoxicity (Müller et al. 2012; Pinhão et al. 2020).

Furthermore, dietary exposure to FB1 may induce several physiological responses in the reproductive system. Regarding the female reproductive function, FB1 has been reported to affect granulosa cell proliferation and steroid production in swine and cattle species (Cortinovis et al. 2014; Albonico et al. 2016). In addition, FB1 has also been reported to have some adverse effects on male reproduction in rats (Voss et al. 1996) and pigs (Harrison et al. 1990). Moreover, many reports have investigated FB1’s effects on sperm quality, mobility, and daily sperm production (Gbore and Egbutue 2008; Minervini et al. 2010; Ewuola and Egbutue 2010a, 2010b). In fact, it has been found that elevated ceramide levels were associated with apoptosis activation in Leydig cells and reduced testosterone production (Lu et al. 2003; Szabó-Fodor et al. 2015).

Despite the highly damaging effects of FB1 on various biological systems, only recently high concern has been
attributed to mycotoxins bioavailability reduction in commodities using *Lactobacillus* strains *in vitro* and *in vivo* studies regarding their abilities to release antifungal substances, which could adsorb or degrade mycotoxins (Hassan and Bullerman 2008; Honoré et al. 2016). In the same way, a recent study from our laboratory showed that treatment with a select strain of *Lactobacillus paracasei* (LP), i.e. LPBEJ01, helped to protect BALB/c mice against hepatic and nephrotic damage that could be induced by FB1 (Ezdini et al. 2020). However, the underlying mechanisms of protection against FB1 nephrotoxicity are still none elucidated.

Therefore, the present study is a follow-up report to examine whether the use of this LP strain could impart protective effects against the adverse effects of FB1 on sperm parameters, daily sperm production, efficiency, testicular oxidative stress generation, and histopathology of the testis.

### 2. Material and methods

#### 2.1. Chemicals and bacteria

Pure Fumonisin B1 (>98% by HPLC) was obtained from Sigma Aldrich and stored in DMSO at −80°C. The working solution of 100 μg/ml of FB1 in phosphate-buffered saline (PBS, pH 7) was prepared from the stock solution. The bacterial strain, *Lactobacillus paracasei* BEJ01 (LP BEJ01), was isolated from Tunisian artisanal butter (Abbès et al. 2013). All the other chemicals used in this study were of analytical grade.

#### 2.2. Experimental animals

In this study, 40 male pubescent mice BALB/c (SEXUAL, St. Doulchard, France) were used (average body weight: 25 ± 0.3 g; age: 6 weeks old). These mice were given standard industrial granules mycotoxin free after check using HPLC and FB1 standard and drinking water *ad libitum*. They were divided into four treatment groups, of 10 mice each. Before treatment, the mice were acclimated for one week under the following conditions: temperature of 23–25°C, relative humidity (45%), and 12 h light/12 h dark cycle. The experimental protocols were approved with the guidelines of the Ethical Committee of the High Institute of Biotechnology of Monastir, Tunisia.

#### 2.3. Experimental design

Mice were divided into four groups and treated for 10 days by oral route using a gastric tube as follows:

- **Group 1:** 200 μl of PBS
- **Group 2:** 200 μl of LP BEJ01 (2 × 10⁹ CFU/ml ~2 mg/kg b.w)
- **Group 3:** 200 μl of FB1 (100 μg/kg b.w corresponding to 10% of the LD₅₀)
- **Group 4:** 200 μl of FB1 (100 μg/kg b.w) and 200 μl of LP BEJ01 (2 × 10⁹ UFC/ml ~2 mg/kg b.w).

The specific doses of LP and FB1 used here were based on our previous report (Ezdini et al. 2020). In fact, FB1 (100 μg/kg b.w) showed a toxic effect on mice. However, no biological damage related to LP was shown. Each day, fresh LP and FB1 stock solutions were prepared to avoid any issue of LP non-viability or potential degradation of FB1. For the fourth group, mice were co-treated with FB1 and LP. The LP and FB1 mixture were prepared accordingly so that the final desired concentrations could be achieved in the fixed 200 μl volume. Dosing occurred at 8 AM each day for 10 days. 24 h after the final treatment, all mice were sacrificed and blood was collected by heart puncture. Plasma was obtained after cold centrifugation serving for the testosterone assay. The epididymis was freshly excised and used for sperm analysis. The right testis was stored at −80°C for the oxidative markers assay while the left testis was taken for the histological study.

#### 2.4. Histology

Testes were immediately fixed in PFA 4%, followed by dehydration in increasing serial concentrations of ethanol then clarification in n-butanol. Samples were embedded in paraffin wax. Sections of 5 μm thick were made using a microtome. Ribbons containing serial sections of the organs were mounted on slides and stained with H&E before microscopic examination.

#### 2.5. Epididymal sperm analysis

**2.5.1. Sperm count and mobility**

The epididymal sperm count was studied using the method of Linder et al. (1986). The epididymis tail was excised and chopped into 1 ml of the pre-incubated RPMI medium at 32°C. The mixture was incubated for 2 min at 32°C and diluted to 1/10. A volume of 20 μl was placed on the hemocytometer cell. All spermatozoids (mobile and non-mobile) were counted in order to determine the percentage of mobility. A microscopic examination was done at a magnification of ×40. 3 replicates from each sample were carried out.

**2.5.2. Daily sperm production**

The daily sperm production (DSP) was assessed following the research protocol of Kyjovska et al. (2013) with some modifications. Indeed, the right testis was harvested after the sacrifice. Its half was homogenized for 60 s in a saline solution containing 0.9% NaCl and 0.05% Triton X-100. Only spermatids of the 14th and 16th stages were resistant to homogenization. To visualize them, staining with the Trypan blue 0.04% in PBS (1 M) was done for 30 min before counting. The spermatids were counted with a light microscope at ×400 magnification. The total elongated spermatids in the right testis were calculated by multiplication with the right testis weight. DSP was calculated by dividing the total of spermatids counted by 4.84 days that spermatids spent to develop into stages 14–16 in mouse species (Oakberg 1956). Then, DSP was divided by the weight of the testis to determine the number of sperm per gram of testis, which corresponds to the efficiency of sperm production.
2.5.3. Sperm viability
The viability was assessed using the Hypo-Osmotic Swelling (HOS) test described by Jeyendran et al. (1984) as a test for evaluating the functional integrity of the sperm membrane. An aliquot of 0.1 ml of epididymal head suspension was added to 1 ml of hypo-osmotic solution and incubated for 1 hour at 37°C.

Microscopic observation was made at ×40 magnification and an enumeration of 200 spermatozoa was done per slide. 3 repetitions of each sample were made. The Viability was expressed as a percentage of viable spermatozoa of the total number counted.

2.5.4. Sperm morphology
For the sperm morphology assessment, a fragment of the epididymis was ground in NaCl saline solution (0.9%) and formalin (10%). The previous suspension was diluted with distilled water for a final volume of 10 ml. Then, 1 ml of eosin 1% was supplemented to the above suspension followed by incubation at room temperature between 40 and 120 min. A drop of the mixture was placed afterward, spread between slide and coverslip serving for microscopic examination, which allowed us to detect morphological abnormalities. The main focus was on the anomalies of the head and tail, the principal parts of the spermatozoids. 300 spermatozoids were counted per slide (3 replicates/sample). Each anomaly was expressed as a percentage of the total number counted.

2.6. Testosterone assay
The plasmatic testosterone concentration was determined by the competitive ELISA technique, according to the Demeditec Testosterone ELISA kit (Demeditec Diagnostics GmbH, REF: DE1559) instructions. The Optical Density (OD) was read at 450 nm and the results were obtained automatically using a 4 Parameter Logistics curve fit by an ELISA reader (λayto, RT-2100C).

2.7. Oxidative stress markers
Testis samples from each group were homogenized into ice and centrifuged on cold for 15 min at 12 000 rpm. The supernatant was stored at −80°C until use, while the cellular debris was thrown. The total protein content was quantified using the Bradford method while we used the bovine serum albumin (BSA) to prepare the standard curve.

2.7.1. Malondialdehyde
Malondialdehyde (MDA) was measured following the method described previously by Yoshioka et al. (1979). A mixture of 100 μl of the testis homogenate, 2 ml of TBA (0.67%), and 800 μl of TCA (20%) was heated in a boiling bath of water for 30 min. The heated samples were cooled and centrifuged at 4000 rpm for 10 min. Then, 3 ml of n-butanol was added to obtain a pink colorant extract. The absorbance of the supernatant was measured at 532 nm in parallel with a blank that contained the entire reagents except for the homogenate. The MDA concentration was expressed as the OD (532 nm) of the molar coefficient of extinction of the TBARS (1.56 × 105).

2.7.2. Conjugated dienes
Conjugated Dienes (CD) as one of the major lipid oxidations was assessed by the method of Esterbauer et al. (1989). In fact, a mixture of 100 μl homogenate, 1 ml of NaCl (0.9%), and 3 ml of chloroform-methanol (2v/1v) was vortexed for 2 min and then centrifuged (4000 rpm, 10 min). The lower phase was transferred into a sterile tube to be evaporated into the oven (70°C). Finally, 1 ml of hexane was added and the OD at 243 nm was measured in parallel with a blank (hexane). The CD quantity was expressed in μmoles/mg of proteins.

2.7.3. Catalase (CAT)
The catalase (CAT) activity was carried out by measuring the decrease in absorbance at 240 nm of the sample for 1 min. The Enzyme activity was expressed in μmoles of H₂O₂/min/mg of proteins, following the protocol of Aebi (1974).

2.7.4. Protein sulfhydryl groups (PSH)
Protein sulfhydryl groups (PSH) activity was assessed as described by Sedlak and Lindsay, (1968). A mixture containing 50 μl of homogenate, 150 μl of 0.2 M Tris (pH = 8.2), 40 μl of 0.02 M EDTA, 790 μl of methanol, and 10 μl of 0.01 M DTNB was incubated for 15 min to be then centrifuged for 14 500 rpm at 4°C for 10 min. After a first record of the absorbance OD1 at 412 nm, samples were supplemented with TCA (5%) in order to precipitate the sulfhydryl proteins. Afterward, 300 μl of the supernatant was incubated for 3 min with the 0.4 M Tris and 0.01 M DTNB. A second absorbance OD2 was done at the same wavelength as OD1. In fact, the PSH content corresponds to subtracting the OD2 (Containing the non-sulfhydryl proteins) from OD1 (the total proteins). The reduced glutathione (GSH) was used to prepare a calibration curve. Finally, the PSH activity was expressed as nmol/mg of protein.

2.7.5. Carbonyl proteins
As previously described by Colombo et al (2016), the carbonyl proteins (CP) were dosed as follows:
A mixture tube containing 200 μl of the homogenate, and 800 μl of DNPH dissolved into HCL (2.5 N), was incubated at room temperature in the dark for 1 h. A vortex every quarter of an hour was made. Then, 1 ml of TCA (20%) was added and the tube on ice was re-incubated for 10 min, after which short centrifugation was done. The supernatant was discarded and the pellet was washed by TCA (10%). New centrifugation was made and the pellet was washed with ethanol/ethyl acetate (1v/1v). The final pellet was dissolved into 500 μl of guanidine hydrochloride (6 M). The absorbance was recorded at 370 nm after incubating the mixture for 10 min at 37°C.
2.8. Statistical analysis

The results of the in vivo study were statistically analyzed with SPSS, IBM 23 using the one-way ANOVA test followed by Tukey as a Post Hoc test. All values were expressed as the mean ± SD and the difference is significant at $p \leq 0.05$.

3. Results

3.1. Histology

Histological examination indicated a normal testicular structure in control mice, as well as in that of the LP-treated group, proved by normal spermatogenesis, and well-organized distribution of cells in the Seminiferous Epithelium (Figure 1). FB1 exposure clearly altered the testicular structure of adult mice, highlighted by the presence of several altered seminiferous tubules, showing hemorrhage, dilation of blood vessels, and infiltration of mononuclear cells into the interstitial space compared to the control animals (Figure 1). No difference in the histological structure was observed in the testis of FB1 + LP treated animals, compared to those of the control, with active spermatogenesis in almost all seminiferous tubules.

In addition, in the testis of control and LP treated mice, very few positive-TUNEL cells could be seen, like spermatogonia (SPG; arrow, Figure 1), spermatocytes (SPC; arrowhead, Figure 1), and spermatids (SPT; Figure 1). On the contrary, FB1 exposure produced a conspicuous increase in the number of TUNEL positive cells in all testicular cells, particularly in the Leydig cells (LC; asterisk, Figure 1). LP treatment partially decreased the FB1-induced apoptosis, since the number of interstitial and germinal apoptotic cells diminished (Figure 1).

3.2. The sperm concentration, viability, motility, and morphology

Table 1 presents the sperm concentration, viability, motility, and morphology of male BALB/c mice in which the FB1 treatment decreased the sperm concentration (101 ± 22.5, $p \leq 0.05$) regarding the concentration of the control (278 ± 30.9, $p \leq 0.05$). Alone (275 ± 27, $p \leq 0.05$) or combined with FB1 (266 ± 22, $p \leq 0.05$), the LP did not affect the sperm concentration.

The spermatozoid viability was also studied (Table 1). The present data showed a slight decrease in the vitality (non-significant) of the treated mice’s sperm. The administration of LP alone or with the FB1 showed 95% of viability as indicated in the control group.

For the motility, which is an important parameter in masculine fertility, our findings showed a decrease in the percentage of sperm motility in the treated mice’s Table 1. The co-treated mice demonstrated similar motility as the control. LP alone revealed a preventive effect on sperm motility affected by FB1 treatment.

The morphological study of the epididymal spermatozoa (Figures 2–4) illustrated that FB1 affected the morphology of the spermatozoa with a high percentage of 85% compared to the other treated and control groups. In particular, a percentage of 60% was recorded as tail abnormalities in parallel with 25% of the head abnormalities (Figures 3 and 4). Interestingly, the administration of LP in combination with FB1 displayed restoration of these malformations caused by FB1-mycotoxin.

![Figure 1](image)

**Figure 1.** Microphotographs section of testicles of control mice (a), exposed to Fumonisins B1 (b), Lactobacillus alone (c) and Combined (LP + FB1) (d) for 10 days stained with hematoxylin&eosin (magnification: ×400). AST: Altered seminiferous tubules; H: showing hemorrhage; BVD: dilation of blood vessels; L: infiltration of mononuclear cells.

| Group       | Control | FB1 | LP    | FB1 + LP |
|-------------|---------|-----|-------|----------|
| Sperm parameters |        |     |       |          |
| Concentration ($\times 10^6$/ml) | 278 ± 30.9 | 101 ± 22.5 | 275 ± 27 | 266 ± 22 |
| Mobility (%) | 58 ± 8  | 19 ± 3 | 63 ± 4 | 62 ± 8  |
| Viability (%) | 96 ± 2 | 72 ± 13 | 96 ± 1 | 94 ± 2  |
| Abnormal Morphology (%) | 13 ± 2.4 | 85 ± 1.7 | 10 ± 3 | 17 ± 3.5 |

Data are expressed as mean ± SD from 6 mice per group. *Significantly different from the control ($p < 0.05$). †Not significantly different from the control ($p < 0.05$).
3.3. DSP and DSP efficiency

The DSP and DSP efficiency values are mentioned in Table 2. Results revealed that there is no difference between the control (25 ± 2, \( p \leq 0.05 \)) and LP groups (26 ± 2, \( p \leq 0.05 \)). Mice exposed to FB1 induced a significant decrease (8 ± 2, \( p \leq 0.05 \)) in the DSP and DSP efficiency (106 ± 10, \( p \leq 0.05 \)) compared to the control group (359 ± 39, \( p \leq 0.05 \)). The co-treatment of LP with FB1 justified a powerful effect to restore these altered parameters in the treated group.

3.4. Testosterone level

Testosterone quantification by Elisa kit in the control, FB1, LP, and LP combined with FB1 groups is demonstrated in Figure 5. The plasmatic concentration observed in the LP group was similar to the control one. The exposure of animals to FB1 caused a notable decline in testosterone levels. Combined with the FB1, the LP strain showed a potential effect to reverse the FB1 disturbance.
3.5. Oxidative stress

The results of the oxidative stress biomarkers Table 3 revealed that treatment of mice with LP alone did not alter the balance of the oxidative stress markers as compared with the control. The FB1 administration generated oxidative stress via the increase of MDA, CD, and PC accompanied by a decline in CAT and PSH activities.

Inversely, the companied treatment with FB1 plus LP improved the failure of the oxidant system caused by FB1.

Table 2. Testicular daily sperm production and efficiency on male balb/c mice.

| Group          | Control | FB1 | LP | FB1 + LP |
|----------------|---------|-----|----|----------|
| DSP/testis     | 25 ± 2  | 8 ± 2<sup>a</sup> | 26 ± 2<sup>b</sup> | 23 ± 1<sup>b</sup> |
| Efficiency     | 359 ± 39 | 106 ± 10<sup>a</sup> | 447 ± 14<sup>b</sup> | 415 ± 28<sup>b</sup> |

Data are expressed as mean ± SD from 6 mice per group. <sup>a</sup>Significantly different from the control (<i>p</i> < 0.05). <sup>b</sup>Not significantly different from the control (<i>p</i> < 0.05).

Discussion

Scientific research about mycotoxins and particularly in FB1 has received greater attention because of the significant economic losses that crops would face by a fungal infestation. It is therefore important to develop several strategies to reduce its spread in the matrix used in the alimentary chain for both human food and animal feed. In fact, numerous harmful effects related to FB1-mycotoxin have been discovered. They have been found to be carcinogenic for humans (IARC 2002), immunotoxic (Stoev et al. 2012), neurotoxic (Suarez et al. 2012), and genotoxic (Chuturgoon et al. 2014). In contrast, only a few reports about its infestation in the male reproductive system are available. Consequently, the purpose of this study was to evaluate the effect of FB1 on BALB/c mice sperm quality, testosterone level, oxidative stress induction as well as histological alterations in the reproductive organs. Furthermore, based on the consumer demand to benefit from safe food devoid of mycotoxins, we evaluated the Lactobacillus paracasei BEJ01, to mitigate FB1-reprotoxicities. The mentioned bacteria were already used in our previous study and by itself was safe and disclosed potential protective effects against general toxicities (Abbès et al. 2016; Ezdini et al. 2020).

In this study, mice exposed to FB1 for 10 days through an oral route showed a positive correlation between FB1 treatment and the reproductive system disturbance. Our results revealed testicular alterations marked by the presence of such debris and vacuolization in the lamina of the seminiferous tubules. A wide interstitial space was also observed.

These findings were in accordance with those of Abdel-Wahab et al (2018) who reported that rats treated with FB1 (100 mg/kg b.w) showed vacuolation (V) in seminiferous tubules lumen, distorted spermatocytes, and the interstitium containing vacuoles and edematous spaces. These structural abnormalities may be attributed not only due to the toxin accumulation in the testis but also due to the indirect effect of FB1 on the disruption of the cell membrane as a sphingolipids inhibitor.

Furthermore, the present data highlights the effect of this toxin on sperm parameters. First, the concentration of sperm was linked to spermatogenesis occurrence in the seminiferous tubule and associated with the maturation of germ cells. As a consequence of the abnormalities located in the testis as described by the histological micrographs (Figure 1), sperm concentration, daily sperm production, and efficiency significantly declined. The suggested mechanism of FB1 toward the spermatogenesis process was probably through its potential to elevate the sphingolipids contents, which was associated with the apoptosis event. Here, we hypothesized that a germ cell apoptosis event occurs in the testicular epithelium as an effect of FB1 treatment leading to a reduction of the germ cell population, and hence, a reduction in the studied parameters of the sperm quantity in both testis and epididymis. These findings could support the results suggested by Szabó-Fodor et al. (2015) in which the disturbance of meiosis and mitosis of the germinal epithelial cells induced by FB1 alone or combined with ZEN and DON in rabbit buck was documented.

Table 3. Testicular malondialdehyde (MDA), carbonyl proteins (CP), catalase (CAT), protein sulfhydryl groups (PSH), conjugated dienes (CD) levels issued from control, treated with FB1 alone, LP alone or combined with FB1.

| Testis     | Control | FB1   | LP    | FB1 + LP |
|------------|---------|-------|-------|----------|
| MDA (nmoles/mg of proteins) | 0.22 ± 0.04 | 0.54 ± 0.03<sup>a</sup> | 0.22 ± 0.04<sup>b</sup> | 0.18 ± 0.05<sup>b</sup> |
| CD (μmoles/mg of proteins)  | 1.18 ± 0.16 | 2.24 ± 0.2<sup>a</sup> | 1.28 ± 0.2<sup>b</sup> | 1.38 ± 0.07<sup>b</sup> |
| CAT (μmoles of H<sub>2</sub>O<sub>2</sub>/min/mg of proteins) | 0.24 ± 0.02 | 0.07 ± 0.02<sup>a</sup> | 0.18 ± 0.01<sup>b</sup> | 0.23 ± 0.01<sup>b</sup> |
| PSH (nmoles/mg of proteins) | 7.92 ± 0.55 | 1.8 ± 0.2<sup>b</sup> | 6.58 ± 0.3<sup>b</sup> | 7.97 ± 0.5<sup>b</sup> |
| CP (μmoles/mg of proteins)  | 0.01 ± 0.002 | 0.023 ± 0.003<sup>a</sup> | 0.008 ± 0.002<sup>b</sup> | 0.01 ± 0.001<sup>b</sup> |

Data are expressed as mean ± SD from 6 mice per group. <sup>a</sup>Significantly different from the control (<i>p</i> < 0.05). <sup>b</sup>Not significantly different from the control (<i>p</i> < 0.05).

Figure 5. Plasmatic testosterone level of control, treated with FB1 alone, LP alone and FB1 + LP. Data are expressed as mean ± SD from 6 mice per group. <sup>a</sup>Significantly different from the control (<i>p</i> < 0.05). <sup>b</sup>Not significantly different from the control (<i>p</i> < 0.05).
Moreover, a slight reduction in sperm vitality was recorded in the treated mice. In addition, a sharp decline in motility was observed. In the main context, our results were supported by several studies. In fact, Minervini et al. (2010) reported that FB1 reduced the total and progressive motility of equine spermatozoa. A similar phenomenon that affected spermatogenesis and sperm parameters were reported as a result of a diet containing more than 5 mg/kg of FB1 in wild boar (Gbore and Egbunike 2008). Likewise, livestock was affected as shown by Ewuola and Egbunike (2010a). As a matter of fact, Spermatic mass, motility, and viability of the rabbit’s semen declined in correlation with an increase in the dietary of FB1. The mobility was sensitive to the morphology of the spermatozoa. Actually, the mice’s exposure to FB1 revealed a deleterious effect on sperm morphology; in particular, 60% of tail abnormalities and 25% of the head were recorded. The dominance of the tail abnormalities revealed the decline in sperm motility mentioned earlier. How could FB1 significantly disrupt spermatogenesis and spermatozoa morphology? Two mechanisms were suggested: the first one was the reduction of the hormonal level, especially the testosterone quantity. Similarly, Abdel-Waheb et al. (2018) stated the sensitivity of the testosterone level in the FB1 treatment alone or combined with AFB1. This hormonal disruption may be described by the capability of FB1 to adjust cholesterol and lipid homeostasis in the absence of LXR in the liver (Régnier et al. 2019). An involvement of LPCAT3, a sphingolipid enzyme, was suggested in FB1 cholesterol modulation (Rong et al. 2013; Wang et al. 2016). In the same vein, FB1 decreased LDLR and consequently inhibited the cholesterol influx (Abdul and Chutgurgoon 2021).

Here, we suggest as a hypothesis that the established mechanism of FB1 affecting the cholesterol pathway in the liver could be adopted as the same in the Leydig cells via the absence of reports studying this detailed mechanism in the testis. Secondly, we hypothesize that the ROS levels and their antioxidant defense imbalance led to oxidative damage in the spermatozoa (Fraczek and Kurpisz 2005). Consequently, we focused on measuring the oxidative stress occurring in the testis upon FB1 exposure. Our data revealed that the FB1 treatment elevated the MDA, CD, and protein carbonyl levels, while, catalase and PSH activities decreased.

The oxidative stress involved through the free radicals and peroxides resulted in the damage of the sperm membrane, and decreased sperm motility. Thus, the displayed abnormalities and impaired motility of sperm could be a result of the abnormal functionality of the mitochondria (Chai et al. 2017). Several pathways may be involved in the effect of FB1 on the mitochondria generating ROS. Due to its analogy to sphingolipids, FB1 was able to increase the polyunsaturated fatty acids content, trigger the mitochondrial permeability, and then the induction of apoptosis.

These key mechanisms proposed were previously indicated by (Aitken et al. 2012) who reported that ROS generation drives the spermatozoa along the intrinsic apoptotic cascade by the loss of Mitochondrial Membrane Potential, leading to DNA adduct formation and fragmentation, and ultimately cell death. In the main content, Nowicka-Bauer and Nixon (2020) observed that ROS deregulated sperm bioenergetic pathways, along with the structural and signaling machinery of the sperm tail confirming 60% of tail abnormalities also found in this study.

The supplementation of Lactobacillus strain alone did not cause any sign of toxicity and all the studied parameters showed normal spermatogenesis as well as good sperm quality in comparison with the normal group. Thus, several reports proved that Lactobacillus strains benefit from a wide range of advantageous effects. In fact, in previous studies, Lactobacillus was demonstrated to have a regulatory activity of cholesterol (Huang et al. 2020), and a promoter effect to treat allergic rhinitis (Güvenç et al. 2016). Also, lactic acid bacteria have also shown anti-inflammatory (Abbès et al. 2016), antifungal (Mundula et al. 2019), antimicrobial (Pattani et al. 2013), and antiviral (Lehtoranta et al. 2014) effects.

Supplemented with FB1, our strain showed powerful protective effects against FB1 toxicities on the mouse reproductive system. In fact, the preventive effect of Lactobacillus on FB1 spermatogenesis toxicities was due to its antioxidant activities playing a key role in improving sperm motility via its ion pumps present in the flagella. Sertoli cells benefit also from the powerful antioxidant activities of probiotics boosting the survival and maturation of spermatozoa (Inatomi and Otomaru 2018). Experimentally, several in vitro and in vivo studies showed that probiotics, in particular, Lactobacillus strain enhanced antioxidant enzymes via chelating Fe^{2+} and Cu^{2+} (Lee et al. 2005), reduced ROS imbalance, and by consequence counteracted apoptosis pathways (Yan and Polk 2002; Wu et al. 2019). Likewise, probiotics effectively protect spermatogenic cells, sperm quality, and subsequently protect the reproductive system (Chen et al. 2013; Valcarce et al. 2019; Abasi and Keshmand 2020). Another study has demonstrated a positive correlation between the regulation of cholesterol by probiotics and the plasmatic level of testosterone (Dardmeh et al. 2017).

Finally, another mechanism for the protective role of LP may be involved suggested in probiotics counteracted mycotoxins by sequestration and/or degradation in the gastrointestinal tract (Zhao et al. 2016; Vanhoutte et al. 2017; Ben Salah Abbès et al. 2020).

Conclusion

In summary, our findings showed that the male reproductive system was menaced by FB1, leading to enhance oxidative stress damage, deleterious effects on sperm parameters, reduced testosterone levels, and testicular histological alterations.

LP showed a potential protective effects against the toxic damage of FB1 via several mechanisms, including (i) the antioxidant power of FB1, and (ii) the ability of LP to adsorb or degrade FB1 in the digestive system and then decrease their bioavailability.

Consequently, it would be interesting to investigate whether Lactobacillus metabolites were involved in detoxification processes.
Ethics approval and consent to participate
Experimental protocols were approved with the guidelines of the Ethical Committee of the High Institute of Biotechnology of Monastir, Tunisia.

Animal welfare statement
The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. No ethical approval was required as this is a meta-analysis article with no original research data.

Authors’ contributions
Khawla Ezdini: Methodology, Writing, Original draft, Jalila Ben Salah-Abbes: Data curation, Writing. Hela Belgacem: Visualization, Investigation. Kamel Chaieb: review and editing. Samir Abbès: Supervision, Writing and editing. Bolanle Ojokoh: review and editing.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
This research work was funded by the Tunisian Ministry of Higher Education and Scientific Research Tunisian Ministry of Higher Education and Scientific Research.

ORCID
Samir Abbès http://orcid.org/0000-0002-8466-5063

References
Abasi S, Keshtmand Z. 2020. The effect of probiotic *Bifidobacterium lactis* and *Lactobacillus casei* on sperm maturation in streptozotocin-diabetic rats. ISMJ. 22(6):392–401.

Abbès S, Ben Salah-Abbes J, Jebali R, Younes RB, Oueslati R. 2016. Interaction of aflatoxin B1 and fumonisin B1 in mice causes immunotoxicity and oxidative stress: possible protective role using lactic acid bacteria. J Immunotoxicol. 13(1):46–54.

Abbès S, Ben Salah-Abbes J, Sharafi H, Oueslati R, Moghaddam KA. 2013. *Lactobacillus paracasei* BEJ01 prevents immunotoxic effects during chronic zearalenone exposure in Balb/c mice. Immunopharmacol Immunotoxicol. 35(3):341–348.

Abdel-Wahhab MA, El-Nekeety AA, Hassan NS, Gibrail AA, Abdel-Wahhab KG. 2018. Encapsulation of cinnamon essential oil in whey protein enhances the protective effect against single or combined subchronic toxicity of fumonisin B1 and/or aflatoxin B1 in rats. Environ Sci Pollut Res Int. 25(29):29144–29161.

Abdul NS, Chuturgoon AA. 2021. Fumonisin B1 regulates LDL receptor and ABCA1 expression in an LXR dependent mechanism in liver (HepG2) cells. Toxicon. 190:58–64.

Aebi H. 1974. Catalase. In: Bergmer HU, editor Methods in enzymatic analysis. Vol. 2. New York: Acad; p. 673–684.

Aitken RJ, Whiting S, De Iuliis GN, McClymont S, Mitchell LA, Baker MA. 2012. Electrophilic aldehydes generated by sperm metabolism activate mitochondrial reactive oxygen species generation and apoptosis by targeting succinate dehydrogenase. J Biol Chem. 287(39):33048–33060.

Albonico M, Schütz LF, Caloni F, Cortinovis C, Spicer LJ. 2016. Toxico logical effects of fumonisin B1 alone and in combination with other fusariotoxins on bovine granulosa cells. Toxicon. 118:47–53.

Baldissera MD, Souza CF, da Silva HNP, Henne AS, Duarte FA, da Costa ST, da Silva AS, Baldiessi Filho B. 2020. Diphenyl diselenide modulates splenic purinergic signaling in silver catfish fed diets contaminated with fumonisin B1: an attempt to improve immune and hemostatic responses. Comp Biochem Physiol C Toxic Pharmacol. 227:108624.

Ben Salah-Abbes J, Belgacem H, Ezdini K, Mannai M, Oueslati R, Abbès S. 2020. Immunological effects of AFM1 in experimental subchronic dosing in mice prevented by lactic acid bacteria. Immunopharmacol Immunotoxicol. 42(6):572–581.

Cao C, Zhu X, Li X, Ouyang H, Wang K, Li X. 2020. Assessment of ionic homeostasis imbalance and cytochrome P450 system disturbance in mice during fumonisin B1 (FB1) exposure. Chemosphere. 251:126393.

Chai RR, Chen GW, Shi HJ, O WS, Martin-DeLeon PA, Chen H. 2017. Prohibitin involvement in the generation of mitochondrial superoxide at complex I in human sperm. J Cell Mol Med. 21(1):121–129.

Chen XL, Gong LZ, Xu JX. 2013. Antioxidative activity and protective effect of probiotics against high-fat diet-induced sperm damage in rats. Animal. 7(2):287–292.

Chuturgoon A, Phuludaree A, Moodley D. 2014. Fumonisin B1 induces global DNA hypomethylation in HepG2 cells – an alternative mechanism of action. Toxicology. 315:65–69.

Colombo G, Clerici M, Garavaglia ME, Giustarini D, Rossi R, Milzani A, Dalle-Donne I. 2016. A step-by-step protocol for assaying protein carbonylation in biological samples. J Chromatogr B Analyst Technol Biomed Life Sci. 1019:178–190.

Cortinovis C, Caloni F, Schreiber NB, Spicer LJ. 2014. Effects of fumonisin B1 alone and combined with deoxynivalenol or zearalenone on porcine granulosa cell proliferation and steroid production. Theriogenology. 81(8):1042–1049.

Dardmeh F, Alipour H, Gazerani P, van der Horst G, Brandsborg E, Nielsen H. 2017. Lactobacillus rhamnosus PB01 (DSM 14870) supplementation affects markers of sperm kinetic parameters in a diet-induced obesity mice model. PLos ONE. 12(10):e0185964.

Dellaflora L, Galverna G, D’Asta C. 2018. Mechanisms of fumonisin B1 toxicity: a computational perspective beyond the ceramide synthases inhibition. Chem Res Toxicol. 31(11):1203–1212.

Demirel G, Alpertungu B, Ozden S. 2015. Role of fumonisin B1 on DNA methylation changes in rat kidney and liver cells. Pharmacol Biol. 53(9):1302–1310.

Esterbauer H, Striegel G, Puhl H, Rotheneder S, Milzani A, Gasser D. 1991. Role of vitamin E and carotenoids in preventing oxidation of low density lipoproteins. Ann N Y Acad Sci. 570:254–267.

Ewuola EO, Egbughe N. 2010a. Effects of dietary fumonisin B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. Reproduction. 139(2):439–445.

Ewuola EO, Egbughe N. 2010b. Gonadal and extra-gonadal sperm reserves and sperm production of pubertal rabbits fed dietary fumonisin B1. Animal Reproduction Science. 119(3–4):282–286.

Ezdini K, Salah-Abbes JB, Belgacem H, Mannai M, Abbès S. 2020. *Lactobacillus paracasei* alleviates genotoxicity, oxidative stress status and histopathological damage induced by Fumonisin B1 in BALB/c mice. Toxicon. 185:46–56.

Fraczek M, Kurpisz M. 2005. The redox system in human semen and peroxidative damage of spermatozoa. Postepy Hig Med Dosw (Online). 59:523–534.

Gbore FA. 2010. Brain and hypophyseal acetylcholinesterase activity of pubertal boars fed dietary fumonisin B1. J Anim Physiol Anim Nutr. 94(5):e123–e129.

Gbore FA, Egbughe N. 2008. Testicular and epididymal sperm reserves and sperm production of pubertal boars fed dietary fumonisin B1. Anim Reprod Sci. 105(3–4):392–397.

Güvenç IA, Mutlu NB, Mutlu FS, Eski E, Altenoprak N, Oktener T, Cinigi C. 2016. Do probiotics have a role in the treatment of allergic rhinitis? A comprehensive systematic review and Metaanalysis. Am J Rhinol Allergy. 30(5):e157–e175.
Hassan YI, Bullerman LB. 2008. Antifungal activity of Lactobacillus paracasei subsp. tolerans against Fusarium proliferatum and Fusarium graminearum in a liquid culture setting. Journal of Food Protection. 71(11): 2213–2216.

Honoré AH, Aunsbjerg SD, Ebrahimi P, Thorsen M, Benfeldt C, Knochel S, Skov T. 2016. Metabolic fingerprinting for investigation of antifungal properties of Lactobacillus paracasei. Anal Bioanal Chem. 408(1): 83–96.

Hou L, Yuan X, Le G, Lin Z, Gan F, Li H, Huang K. 2021. Fumonisin B1 induces nephrotoxicity via autophagy mediated by mTORC1 instead of mTORC2 in human renal tubule epithelial cells. Food Chem Toxicol. 149:112037.

Huang Y, Xiao Y, Song L, Chu Q, Zhu S, Ren Z, Li X. 2020. Effects of Lactobacillus paragasseri Y20 on cholesterol-lowering, intestinal microbiota and liver metabolism in rats with hypercholesterolaemia. Wei Sheng Yan Jiu. 49(4):574–579.

IARC. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol. 82. Lyon (France): IARC.

Inatomi T, Otomaru K. 2018. Effect of dietary probiotics on the semen traits and antioxidative activity of male broiler breeders. Sci Rep. 8(1): 1–6.

Jedidi I, Mateo EM, Marín P, Jiménez M, Said S, González-Jaén MT. 2021. Contamination of wheat, barley and maize seeds with toxigenic Fusarium species and their mycotoxins in Tunisia. Journal of AOAC International. 104(4):959–967.

Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. 1994. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil. 70(1):219–228.

Kim SH, Singh MP, Sharma C, Kang SC. 2018. Fumonisin B1 facilitates seizures induced by pentylenetetrazol in mice. Neurotoxicol Teratol. 51:61–67.

Régnier M, Polizzi A, Lukowicz C, Smati S, Lasserre F, Lippi Y, Naylies C, Laffitte J, Bétoulières C, Montagner A, et al. 2019. The protective role of liver X receptor (LXR) during fumonisin B1-induced hepatotoxicity. Arch Toxicol. 93(2):505–517.

Rong X, Albert CJ, Hong C, Duerr MA, Chamberlain BT, Darling EJ, Ito A, Gao J, Wang B, Edwards PA, et al. 2013. LXRs regulate ER stress and inflammation through dynamic modulation of membrane phospholipid composition. Cell Metab. 18(5):685–697.

Rumora L, Domijan AM, Grubišić TZ, Peraica M. 2007. Mycotoxin fumonisin B1 alters cellular redox balance and signalling pathways in rat liver and kidney. Toxicology. 242(1–3):31–38.

Sedlak J, Lindsay RH. 1968. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman’s reagent. Anal Biochem. 25(1):192–205.

Stoev SD, Gundasheva D, Zarkov I, Mircheva T, Zapryanova D, Denev S, Mitov Y, Daskalov H, Dutton M, Mwanza M, et al. 2012. Experimental mycotoxic nephropathy in pigs provoked by a mouldy diet containing ochratoxin A and fumonisin B1. Exp Toxicol Pathol. 64(7-8): 733–741.

Suarez L, Felkner M, Brendler JD, Canfield M, Zhu H, Hendricks KA. 2012. Neural tube defects on the Texas-Mexico border: what we’ve learned in the 20 years since the Brownsville cluster. Birth Defects Res A Clin Mol Teratol. 94(11):882–892.

Szabó-Fodor J, Kachlek M, Cseh S, Somoskői B, Szabó A, Bodnár ZB, Tomynos G, Mézes M, Balogh K, Glávits R, et al. 2015. Individual and combined effects of subchronic exposure of three Fusarium toxins (Fumonisin B1, Deoxynivalenol and Zearalenone) in rabbit bucks. Clin Toxicol. 5:415.

Valcarce DG, Riesco MF, Martinez-Vázquez JM, Robles V. 2019. Diet supplemented with antioxidant and anti-inflammatory probiotics improves sperm quality after only one spermatogenic cycle in zebra fish model. Nutrients. 11(4):843.

Vanhouthe I, De Mets L, De Boever M, Uka V, Di Mavungu J, De Saeger S, De Gelder L, Audenaert K. 2017. Microbial detoxification of deoxynivalenol (DON), assessed via a Lemna minor L. bio-assay, through biotransformation to 3-epi-DON and 3-epi-DON-1. Toxins. 9(2):63.

Voss KA, Bacon CW, Norred WP, Chapin RE, Chamberlain WJ, Plattner RD, Meredith FJ. 1996. Studies on the reproductive effects of Fusarium moniliforme culture material in rats and the biodistribution of [14C] fumonisin B1 in pregnant rats. Nat Toxins. 4(1):24–33.

Wang B, Rong X, Duerr MA, Hermanson DJ, Hedde PN, Wong JS, Vallim TQdA, Cravatt BF, Gratton E, Ford DA, et al. 2016. Intestinal phospholipid remodeling is required for dietary-lipid uptake and survival on a high-fat diet. Cell Metab. 23(3):492–504.

Wu Y, Wang B, Xu H, Tang L, Li Y, Gong L, Wang Y, Li W. 2019. Probiotic Bacillus attenuates oxidative stress-induced intestinal injury via p38-mediated autophagy. Front Microbiol. 10:2185.

Yan F, Polk DB. 2002. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem. 277(52): 50959–50965.
Yoshioka T, Kawada K, Shimada T, Mori M. 1979. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 135(3):372–376.

Zhang W, Zhang S, Zhang M, Yang L, Cheng B, Li J, Shan A. 2018. Individual and combined effects of Fusarium toxins on apoptosis in PK15 cells and the protective role of N-acetylcysteine. Food Chem Toxicol. 111:27–43.

Zhao H, Wang X, Zhang J, Zhang J, Zhang B. 2016. The mechanism of Lactobacillus strains for their ability to remove fumonisins B1 and B2. Food Chem Toxicol. 97:40–46.