Lactobacillus Paracasei BEJ01 Protects Against Fumonisin B1-Induced Intestinal Toxicity in Balb/c Mice

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Abstract

Fumonisin B_1 (FB1) is a carcinogenic (Class 2B) mycotoxin produced by *Fusarium* fungi and is responsible for several types of mycotoxicoses in animals and humans. *Lactobacillus paracasei* (LP), as a probiotic, is known to impart a wide range of advantageous effects on host health. The objective of the current study was to evaluate if LP (type BEJ01) isolated from Tunisian artisanal butter, could potentially help protect a host against intestinal alterations caused by exposure to FB1. Here, adult male Balb/c mice were randomly assigned to four groups, i.e., control (vehicle only), treated with FB1 (100 µg/kg BW), LP (2×10^9 CFU/ml [≈ 2 mg/kg BW]) and FB1 (100 µg/kg BW) + LP (2×10^9 CFU/ml) and treated *per os* daily for 10 days. At 24 hr after the final treatment, mice were euthanized and their jejunum harvested for examination of intestinal alterations induced by the FB1. The data showed that a variety of negative effects in jejunal tissue were induced by the FB1, including DNA fragmentation, oxidative stress, apoptotic cell death, and histopathological alterations. The results also showed that co-treatment of LP with FB1 was able to mitigate the harmful FB1 effects. LP alone imparted no damage to jejunal tissues. These results show that apart from potential use as a bio-preservative for extending the shelf-life of food and feeds, LP may also provide a benefit of helping prevent untoward effects from a potent mycotoxin like FB1.

Key Points

- Fumonisin B1 induced jejunal toxicity in balb/c mice
- Lactobacillus prevents jejunal toxicity
- Lactobacillus could be used as a candidate for mycotoxins detoxification

Introduction

Maintenance of a safe food-chain is vital to all living beings. Unfortunately, many important foodstuffs (e.g., grains, fruits, vegetables) often act as major vectors for the trans-mission of environmental contaminants, including molds and their associated mycotoxins. The latter agents can cause a variety of mycotoxicoses (mycotoxin-induced pathologies/toxicities) that in turn impact on public health, and farm animal productivity.

Fumonisin B1 (FB1) produced by *Fusarium moniliforme* is classified by IARC (International Agency for Research on Cancer) as a Group 2B carcinogen to humans (IARC 2012). FB1 exposure is associated with neural tube defects in infants (Gelineau-Van Waes et al. 2005) and oesophageal cancer (Alizadeh et al. 2012). Other data has shown that FB1 can also impart a variety of adverse effects in several animal hosts, including hepatotoxicity (Régnier et al. 2017), nephrotoxicity (Voss et al. 2011) repro-toxicity (Albonico et al. 2016) and neurotoxicity (Bódi et al. 2020). With regard to the latter, it is known that FB1 exposure can cause with leukoencephalomalacia in equids (Marasas et al. 1988), pulmonary edema syndrome in porcine hosts (Ross et al. 1990), and spiking disease in chickens (Ledoux et al. 1992). Several studies have shown that toxicity of FB1 is mediated in part by an interruption of sphingolipid

pathways (Merrill et al. 1993). One study showed FB1 was able to inhibit ceramide synthases such that host tissues contained elevated sphinganine and decreased ceramide levels (Yoo et al. 1996).

In the gut, ingested FB1 can cause intestinal lesions by imparting deleterious effects on both intestinal and local epithelial cells. In rodents, FB1 was able to create disturbances in the intestinal gap junctions (Basso et al. 2013). Regarding its effect on gut epithelial cells, FB1 was found to suppress intestinal cell proliferation and damage the integrity of tight junctions, as well as disrupt the expression of mucins (which in turn led to damage of tight junctions/cell barrier functions) (Chen et al. 2019). In humans exposed to FB1, a recent study showed that the mycotoxin caused cytotoxicity, endoplasmic reticulum stress, and apoptotic cell death in gastric epithelial cells (Yu et al. 2020).

To mitigate such types of mycotoxicoses, numerous strategies of detoxification or/and inactivation of these toxins have been suggested. Among these, lactic acid bacteria (LAB) - due to their probiotic properties and reputation as Generally Recognized as Safe (GRAS) - have been a focus of interest for the prevention of mycotoxin toxicities (Hsu et al. 2018; Rogowska et al. 2019). (Ben Taheur et al. 2019) found that a 7-d treatment of *Aspergillus* with LAB inhibited fungal growth and associated mycotoxin production. (Zhao et al. 2016) found *in vitro* that *Lactobacillus* strains had an ability to bind to/'remove' FB1 and another related mycotoxin FB2. A recent study from our laboratory showed that treatment with a select strain of *Lactobacillus paracasei* (LP), i.e., LPBEJ01, helped protect Balb/c mice against hepatic and nephrotic damage that could be inducible by FB1 (Ezdini et al. 2020). Building on those recent findings, the present study was undertaken to examine if use of this LP strain could impart protective effects against jejunal alterations induced by FB1.

Materials And Methods

Chemicals and bacteria

Fumonisin B_1 (FB1) was purchased from Sigma (St. Louis, MO, USA). All other chemicals used here were of pure analytical grade. The *Lactobacillus paracasei* BEJ01 used here was isolated from Tunisian artisanal butter as reported in (Abbès et al. 2013).

Experimental animals

Balb/c mice (male, 8-wk-old, 28-32 g) were obtained from the animal facilities of the Central Pharmacy of Tunis (SIPHAT, Tunis, Tunisia). All mice were housed under controlled conditions in facilities maintained at 20-23°C with a relative humidity of 50% and a 12-hr light/dark cycle. All mice had *ad libitum* access to standard rodent chow (pre-analyzed to assure no mycotoxin content) and filtered tap water. The mice were acclimated 2 wk prior to initiation of any treatments. All procedures here were performed in compliance with the NRC Guide for the Care and Use of Laboratory.

Experimental design

At the time of the treatments, mice were randomly allocated into four treatment groups (10 mice/group) to receive daily gavage: **Group 1:** 200 µl phosphate-buffered saline (PBS, pH 7.2) vehicle; **Group 2:** 200 µl of a solution of LP BEJ01 suspended in PBS (at 2×10^9 UFC/ml [equates to 2 mg /kg BW/dosing]); **Group 3:** 200 µl of a solution of FB1 in PBS (100 µg/kg BW/dosing); **or, Group 4:** 200 µl of a solution containing both FB1 (100 µg/kg) and LP BEJ01 (2×10^9 UFC/ml). The particular concentrations of LP and FB1 used were based on results from Ezdini et al. (2020) where these levels were shown to induce toxicity for FB1 and no biological damage related for the LP. Each day, fresh LP and FB1 stock solutions were prepared to avoid any issue of LP non-viability or potential degradation of the FB1. For Group 4, the LP and FB1 stocks were prepared accordingly so that the final desired concentrations could be achieved in the fixed 200 µl volume. Dosing each day occurred at 8 AM. Dosing was done for 10 days. At 24 hr after the final treatment, all mice were euthanized by cervical dislocation. At necropsy, the entire jejunum was isolated. This material was then either immediately stored at -80°C (for use in subsequent assays below) or a section was removed for histopathological analyses (see below).

Histopathology

A small piece of isolated jejunum was immediately fixed in 4% paraformaldehyde, then placed in paraffin, sectioned to 5-µm using a cryostat, and affixed to slides. The slides then underwent serial dehydration with ascending concentrations of ethanol (70, 80, 90, 96, and 100%) followed by two successive baths in n-butanol to permit clarification. The sections were then stained with hematoxylin and eosin (H&E). A minimum of five slides were prepared and examined for each mouse. To assess presence/extent of any alterations, slides were examined using a Zeiss light microscope at 100X and 400X magnifications (Zeiss, Tokyo, Japan). Among endpoints evaluated were lymphocyte accumulation, presence of glandular crypts, enterocyte loss, as well as villous height length. Values for each endpoint were then averaged across the five samples/mouse before averaging across all mice in each group. All data were analyzed using Image J software (NIH, Bethesda, MD).

DNA ladder assay

Total genomic DNA from each jejunum sample was extracted using the method in (Lu et al. 2002). In brief, a 50 µg piece of frozen sample was thawed and then digested over-night at 37°C in a solution of PBS containing proteinase K (20 mg/ml). The sample was then treated with 5 M NaCl solution, held at 37°C for 30 min, and centrifuged (10 min, 10000 rpm, room temperature). Resulting supernatant was collected, RNase (10 U/ml final concentration) was added, and the solution incubated 30 min at 37°C. The solution was then extracted three times with phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v). The DNA that remained in the final upper-phase was precipitated in the cold using absolute ethanol. The isolated total DNA was pelleted by centrifugation (10 min, 10000 rpm, 4°C), washed twice with 70% ethanol, and then dried for 30 min in a speed-vac. The final material isolated was dissolved in ultra-pure water and its concentration determined spectrophotometrically. In addition, an aliquot was removed and visualization of any fragmented DNA assessed by resolution of a 20-µg sample over a 1% agarose gel

that was subsequently stained with ethidium bromide (0.05 mg/ml). A 1000-bp ladder was run in parallel in each gel as a molecular marker.

Semi-quantitative PCR

Pre-/pro-apoptotic gene expression in the jejunal tissues was quantified using semi-quantitative PCR (Raben et al. 1996). In brief, RNA was isolated from a 50 µg thawed tissue sample using TRIzol reagent (Sigma). All initial RNA extracts were treated with 10 U/ml DNase to avoid a presence of DNA contaminants. Following isolation, the purity and quality of each isolate was confirmed by optical density measures at 260 and 280 nm. Reverse trans-cription (for cDNA) was then done by combining an aliquot containing 20 µg total RNA with 2 µl 10× PCR buffer (Promega, Madison, WI, USA), 2 µl dNTP (2.5 mM each, Biotech, Union City, CA, USA), 0.5 µl Taq DNA polymerase (5 U/µl; Promega), 0.2 µl forward/reverse primers (50 pmole; Polygene, Rümlang, Switzerland), and 0.1% diethyl-pyrocarbonate water, for a final total volume of 20 µl. The resulting PCR products were then analyzed via 1.5 % agarose gel electrophoresis. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) - used as the internal control - was amplified in parallel. The primers used here are listed in Table 1.

Western blot

To analyze expression of the pre-/pro-apoptotic proteins Bax, BCL-2, caspase-3, caspase-9, and p53 in the jejunal samples, ≈ 100 mg thawed tissue was placed in 2 ml RIPA assay buffer containing protease inhibitor (Abcam, London, UK). Each sample was homo-genized under constant cold agitation for 2 hr, then centrifuged (12000 rpm, 15 min, 4°C), and the resulting supernatant collected. Isolate protein concentration was then quantified using a BSA protein assay (Merril 2000). Thereafter, 35 µg protein extract/sample was loaded into wells in a 15% SDS-PAGE gel, the materials resolved, and then all proteins electrotransferred to a PVDF (polyvinyldene difluoride) membrane. A separate gel/membrane was generated for each protein of interest; this allowed for avoiding issues of cross-fluorescence and loss of antigen had membrane stripping steps been employed.

Non-specific binding sites on each membrane were blocked by incubating for 15 min at 37°C in a solution of 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST). Each membrane was then incubated overnight at 4°C in TBST containing a specific primary antibody (i.e., rabbit anti-mouse-bax, -caspase-3 or -9, -bcl2, or -p53, in each case along with anti-GADPH). Each membrane was then washed gently three times with TBST before being placed in TBST containing horseradish peroxidase-conjugated anti-rabbit secondary antibody and incubated at 4°C for 30 min. All antibodies were purchased from Sigma (St. Louis, MO, USA). The membranes were then placed in a solution of TBST containing chemiluminescent substrate (Sigma–Aldrich, Milan, Italy) to permit protein detection and quantification. All gel images were acquired with a CCD camera and all data analyzed with Image J software. Ultimately, levels of each protein were normalized to levels of GADPH in each sample.

Oxidative stress markers in the jejuna mitochondria

Jejunal mitochondria were isolated using the method of (APRILLE et al. 1977). In brief, thawed tissue samples (≈ 0.5 mg/mouse) were placed in a volume (i.e., 5 ml/g tissue) of extraction buffer (225 mM mannitol, 75 mM sucrose, 0.05 mM EDTA, in 10 mM Tris-HCl [pH 7.4]). The sample was then homogenized and then centrifuged (600 x g, 5 min, 4°C) to remove cell debris and the cell nuclear fraction. Resulting supernatant was isolated and centrifuged at (8800 x g, 10 min, 4°C) to sediment all mitochondria present. The resulting pellet was washed twice with PBS and stored at -80°C until analyzed for levels of catalase (CAT), protein sulfhydryl (PSH), malondialdehyde (MDA), conjugated diene (CD), and carbonyl protein (CP), using standard methods for each determination. All measures were in turn normalized to the total protein content in the isolated mitochondria (assessed using Bradford method).

Statistical analysis

All values shown are means ± SD. All statistical analyses were performed using an SPSS software package (v.22.0. IBM Corp., Armonk, NY, USA). Differences between groups for a given endpoint were compared using a one-way analysis of variance (ANOVA) test followed by a Tukey's test. Differences were considered significant at $p \leq 0.05$.

Results

DNA fragmentation

The electrophoresis data shown in Figure 1 indicate that treatment with FB1 caused DNA fragmentation (smear) in jejunal samples from the mice. Exposure to the LP strain alone did not affect intestinal DNA. Combined with the FB1, LP attenuated the damaging effect induced by the FB1 in the intestine.

Pre-/pro-apoptotic gene expression

The expression of select pre-/pro-apoptotic genes such as *Bax*, *bcl-2*, *casp-3*, *casp-9*, and *p53* in jejunal samples was analyzed (Figure 2). The results show host treatment with FB1 affected expression of both pre- and pro-apoptotic genes in the small intestine. Indeed, down-regulation of *bcl-2* (inhibitor of apoptosis; 2.5-fold vs. in control mice tissues) expression was evident. In contrast, there was a concomitant significant up-regulation of Bax, casp-3, casp-9, and p53 expression in the tissues of these hosts, i.e., 1.8-, 2.3-, 2.4-, and 2.8-fold, respectively, relative to control mice tissue values. Exposure to LP alone did not impact on expression of any of the genes evaluated. Co-treatment with FB1 and the LP strain appeared to prevent any of the changes noted with the mycotoxin alone, with expression levels being on par with that of the control mice.

Western blotting analysis

Jejunal expression of select pre-/pro-apoptotic proteins like Bax, BCL-2, caspase-3, caspase-9, and p53 were also analyzed (Figure 3). Results indicate that FB1 treatment resulted in significantly increased Bax, caspase-3 and -9, and p53 levels in isolated jejunal tissues compared to corresponding values in control mouse tissues. Mean levels of increase were 1.8-, 1.9-, 1.7-, and 1.5-2.0-fold, respectively, relative to values

seen in the control tissues. Again, in contrast, expression of the bcl-2 inhibitor of apoptosis was significantly decreased (0.5-fold vs. control levels) by the FB1 treatment. As with the gene outcomes, exposure to LP alone did not impact on expression of any of the proteins evaluated. Co-treatment with FB1 and the probiotic appeared to prevent any of the changes noted with the mycotoxin alone, with expression levels being on par with that of the control mice.

Oxidative stress in mitochondria

Oxidative stress marker assays for the jejunal mitochondria (Table 2) revealed an imbalance among pro-/anti-oxidant enzymes, indicating mitochondrial oxidative stress in the jejunum caused by FB1 treatment. Specifically, the data showed a significant increase in MDA, conjugated dienes, and carbonyl proteins levels concomitant with a sharp decline in catalase and PSH values as a consequence of the toxin treatment. Treatment with LP alone or combined with the FB1 did not result in significant change in these parameters compared to in the control mice.

Histology

Light microscopy analyses of isolated tissue (Figure 4) showed that in control mice, there was normal jejunal histology, i.e., normal mucosa lined by simple columnar epithelium containing both finger-like villi and crypts of Lieberkuhn. The major cells in the epithelium were prism cells with striated plateau (arrow) and goblet cells. In mice treated with LP alone, the jejunum contained villi with normal architecture as well as what seemed to be a normal number of enterocytes and goblet cells. In comparison, sections from mice treated with FB1 alone revealed several modifications, mainly in the intestinal mucosa (Figure 5) and an atrophy/decrease in the height of their villi (Figure 6). Moderate loss of glandular crypts at the base of the villi was also noted. There was also an overall loss of enterocytes in the villi, an accumulation of lymphocytes (generally, cytotoxic lymphocytes) in the intestine (Figure 7), as well as the appearance of large numbers of plasmocytes in the chorion. Co-administration of LP with the FB1 appeared to mitigate this damage to the jejunum, though there were some still changes noted (relative to in control mice tissues) (see Figure 4).

Discussion

Mycotoxins are persistent food contaminants in several foodstuffs and exposures to them are associated with harmful effects in animals and humans. Among mycotoxins routinely encountered, Fumonisin B_1 (FB1) is one of the more potent, with a capacity to induce a variety of hepatotoxic (Abdel-Wahhab et al. 2018), immunotoxic (Abbès et al. 2016), and neurotoxic (Domijan et al. 2015) effects, as well as to act as a carcinogen (IARC 2012). Thus, developing efficient alternatives to limit the toxicity of this particular toxin is an important goal not just for animal/livestock health, but for the human populations likely exposed as well. In this context, the present study evaluated the possible preventive effect of the probiotic *Lactobacillus paracasei* BEJ01 (isolated from traditional Tunisian butter; (Abbès et al. 2013) against toxic effects of FB1 in the jejunum of mice.

The present results showed that repeated daily exposure (*per os*) to FB1 led to increased jejunal mitochondrial levels of malondialdehyde (MDA), conjugated dienes (CD), and carbonyl proteins (CP), and decreased levels of mitochondrial catalase (CAT, key anti-oxidant enzyme) and total protein sulfhydryl (PSH, reflecting primarily the anti-oxidant glutathione) levels in a mouse model. These findings of increases in mitochondrial oxidative stress were in accordance with previous studies of FB1 toxicity (Theumer et al. 2010; Domijan and Abramov 2011; Abbès et al. 2016; Khan et al. 2018). One possible explanation for the presence of these stress/oxidative markers was an over-production of reactive oxygen species (ROS) secondary to a blockage in mitochondrial membrane potentials (Domijan and Abramov 2011). These types of oxidative stresses might also help explain the DNA fragmentation resulting from FB1 exposure here, an event consistent with earlier findings on FB1 genotoxicity (FAO/WHO 2001; Abbès et al. 2016). Those findings were also in line with data of other studies that showed that exposure to FB1 created DNA lesions in human peripheral blood lymphocytes (Domijan et al. 2015) and gave rise to apparent increased apoptosis [i.e., DNA fragmentation, chromatin condensation, and hyperchromasia] in murine hepatocytes (Ribeiro et al. 2010).

The current study also showed that FB1 exposure disrupted expression of select pre- and pro-apoptotic genes and their resulting proteins in the jejunum of the mice. The results indicated that FB1 alone up-regulated Bax, casp-3, casp-9 and p53, and down-regulated Bcl-2, gene and protein expression. Such outcomes were in keeping with the findings by (Kim et al. 2018) who noted an increase in Bax, caspase-3 and p53 expression (and a decrease in Bcl-2 expression) in the colon of mice exposed to FB1. These types of changes portend the onset/progression of pathologies in the affected tissue. Specifically, FB1-induced increases in Bax and caspase-3 expression in conjunction with decreased Bcl-2 expression led to an apoptotic cell death in rat liver cells (i.e., BRL line) (Wang et al. 2020). Similarly, (Klarić et al. 2008) showed that FB1 exposure of PK15 kidney cells *in vitro* led to increased caspase-3 activity and apoptosis. Additionally, (Zhang et al. 2018) showed that exposure to a combination of toxins (FB1-ZEN-DON) caused increased expression of p53, Caspase-3 and Caspase-9 in porcine kidney cells (PK15). From these studies and the current data, it is reasonable to conclude that repeated exposures to FB1 were likely to impact on many of the cells that reside within the jejunum, with one key outcome being an increase in the appearance of apoptotic/dead cells among these cell types.

The histological changes noted in the jejunal samples showed that the FB1 caused decreases in villi height, along with complete loss of/fusion among villi. These types of findings are in line with those noted by (Basso et al. 2013) who demonstrated that FB1 alone impacted jejunum villi. In that study, the authors believed that these structural modifications were likely a result of changes induced in intestinal gap junctions and in E-cadherin expression. Whether or not the histological changes noted here were secondary to increases in apoptotic events within cells of the jejunum remain to be confirmed.

Though the findings here on FB1 toxicity were primarily confirmatory of earlier ones in the above noted studies, the present study also showed that host co-treatment with the LPBEJ01 strain appeared to mitigate many of the toxicities inducible by FB1 in the jejunum of the test mice. Based on findings from several other studies using *Lactobacillus* strains, it could be that the anti-oxidative effects (Düz et al.

2020; Zhou et al. 2020) imparted by the microorganism were sufficient to prevent the expected damage to gut cells expected to be induced by the FB1. Conversely (or even concurrently), the ability of some *Lactobacillus* strains to bind myco-toxins (Kankaanpää et al. 2000; Jebali et al. 2018) during the co-treatments could be the under-lying mechanism of protection being observed here. Regardless of which proffered mechanism of effect is correct (or predominant), it still remains to be determined if use of the LPBEJ01 after the fact (i.e., after a host is exposed to FB1) also provides any protective effect to a host. If it turns out *not to be the case*, this would strengthen any recommendation for use of LPBEJ01 as a prophylactic to prevent human toxicities from exposure to this organism or its mycotoxin products, especially in areas of the world where *Fusarium* infestations might be manifest year-round (tropical regions, etc.).

Declarations

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Author contribution statement

KE: design methodology and writing the original draft. JB: curate data and writing. HB: visualized the data; KCh: review and editing. SA: overall project supervision, writing and editing.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

Ethical review

This study does not involve any human testing. This study was approved by the Institutional Review Board of Higher Institute of Biotechnology, Monastir University.

Data availability

All data generated or analyzed during this study are included in this manuscript.

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Tables

Table 1. Primers used in the studies.

Gene	Primer Sequences
Caspase-3	5'-AAATTCAAGGGACGGGTCAT-3'
Caspase-9	5'-ATTGACACAATACACGGGATCTGT-3'
Bax	5'-CATCCCCACAATACCAACAAT-3'
Bcl-2	5'-CACAAAGATGGTCACTGTCTGC-3'
GDPH	5'-TTGTGGCCTTCTTTGAGTTCG-3'

Table 2. Oxidative stress parameters in jejunal mitochondria.

Parameter	Control	FB1	LP	FB1+LP
MDA (nmole/mg protein)	[a]0.21 ± 0.03	[b]0.48 ± 0.05	[a]0.21 ± 0.03	[a]0.24 ± 0.0
CD (nmole/mg protein)	[a]0.41 ± 0.06	[b]0.84 ± 0.09	[a]0.42 ± 0.05	[a]0.43 ± 0.0
CAT (nmole/min/mg protein)	[a]0.21 ± 0.02	[b]0.07 ± 0.02	[a]0.20 ± 0.04	[a]0.19 ± 0.0
PSH (nmole/mg protein)	[a]4.47 ± 0.69	[b]0.80 ± 0.20	[a]5.51 ± 0.93	[a]4.70 ± 1.02
CP (nmole/mg protein)	[a]0.01 ± 0.00	[b]0.02 ±0.00	[a]0.01±0.00	[a]0.01 ± 0.00

Means [± SD] in same row without common superscript letter differ significantly ($p < 0.05$).

Figures

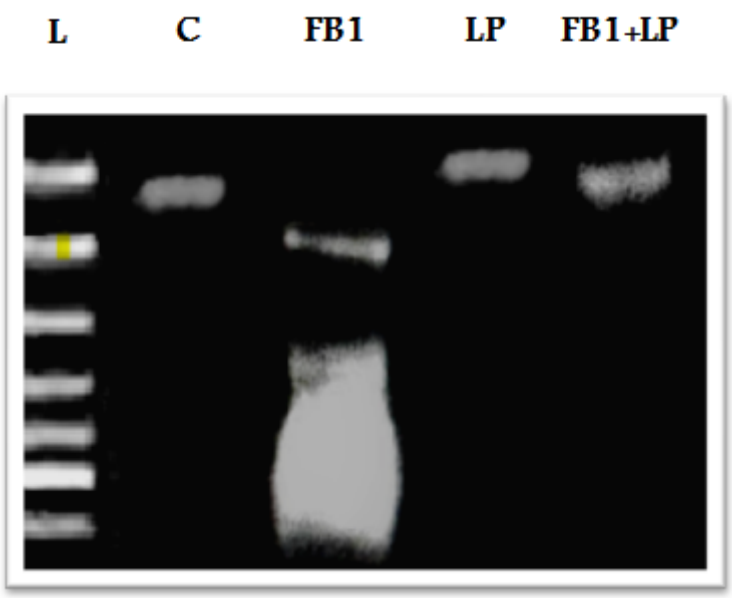

Figure 1

DNA fragmentation in jejunal samples. Samples were analyzed by agarose gel electrophoresis. A representative gel is presented.

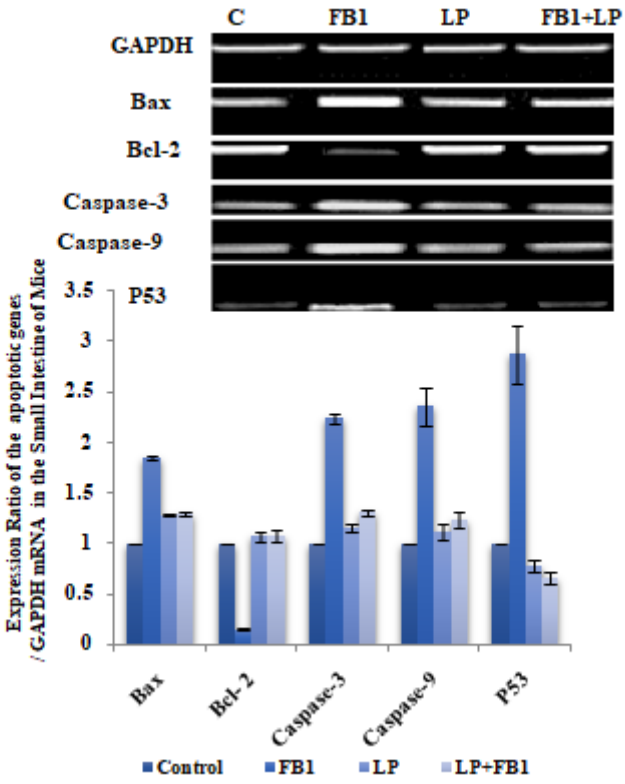

Figure 2

Expression of select pre-/pro-apoptotic genes in jejunal samples. Samples were analyzed by semi-quantitative-PCR. Samples shown are representative blots. Values in bar charts are normalized expressions relative to that of GAPDH. *Value of LP+FB1co-treated group are significantly different from FB1 alone treated group ($p < 0.05$)

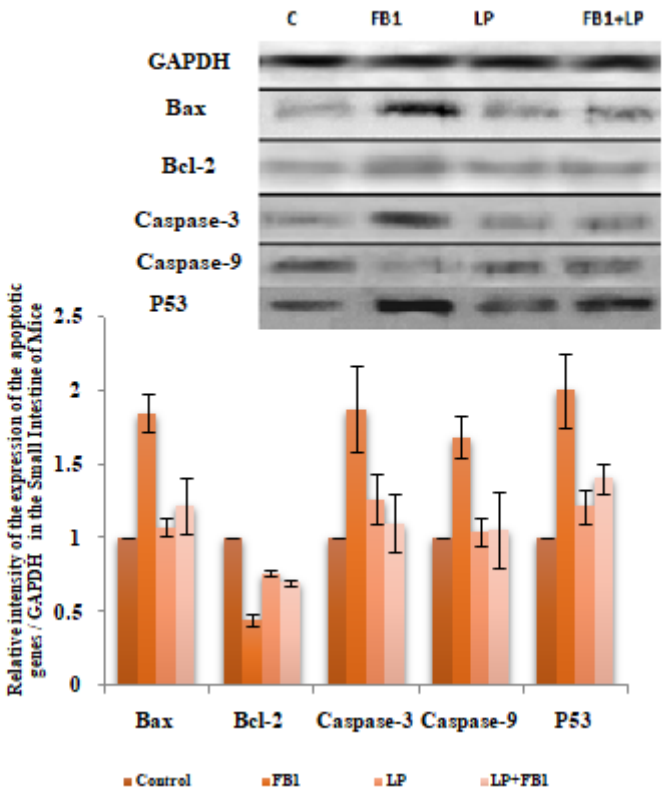

Figure 3

Western blot analysis of select pre-/pro-apoptotic genes in jejunal samples. Samples shown are representative blots. Values in bar charts are normalized expressions relative to that of GAPDH protein. *Value of LP+FB1co-treated group are significantly different from FB1 alone treated group ($p < 0.05$).

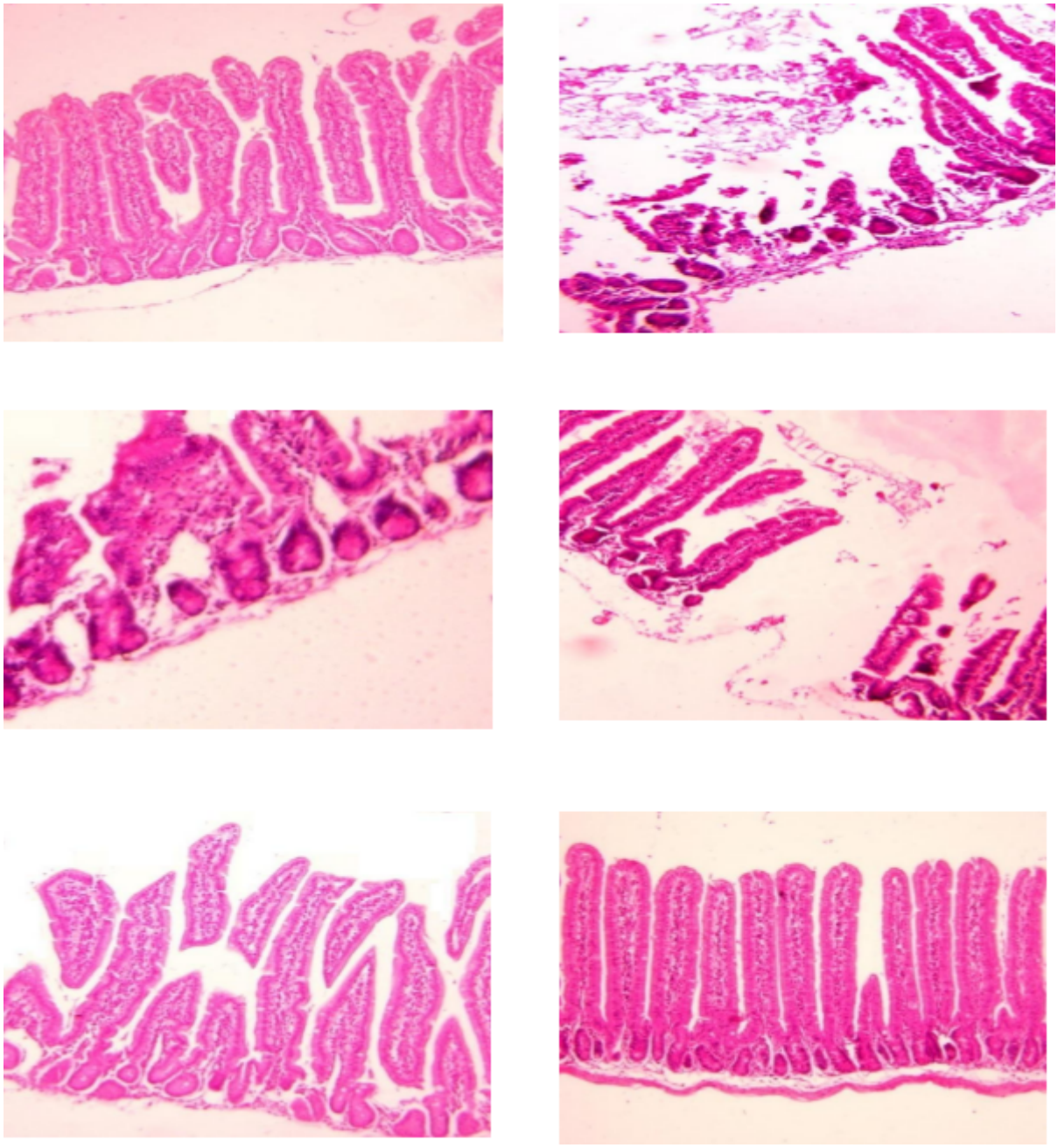

Figure 4

Histological analysis of jejunal sections. Photomicrographs are representative jejunum samples from mice. (A) Control, (B, C, D) FB1 alone, (E) LP alone, (F) LP+FB1. Magnification 100X. V: villi, C: crypt, F: fusion.

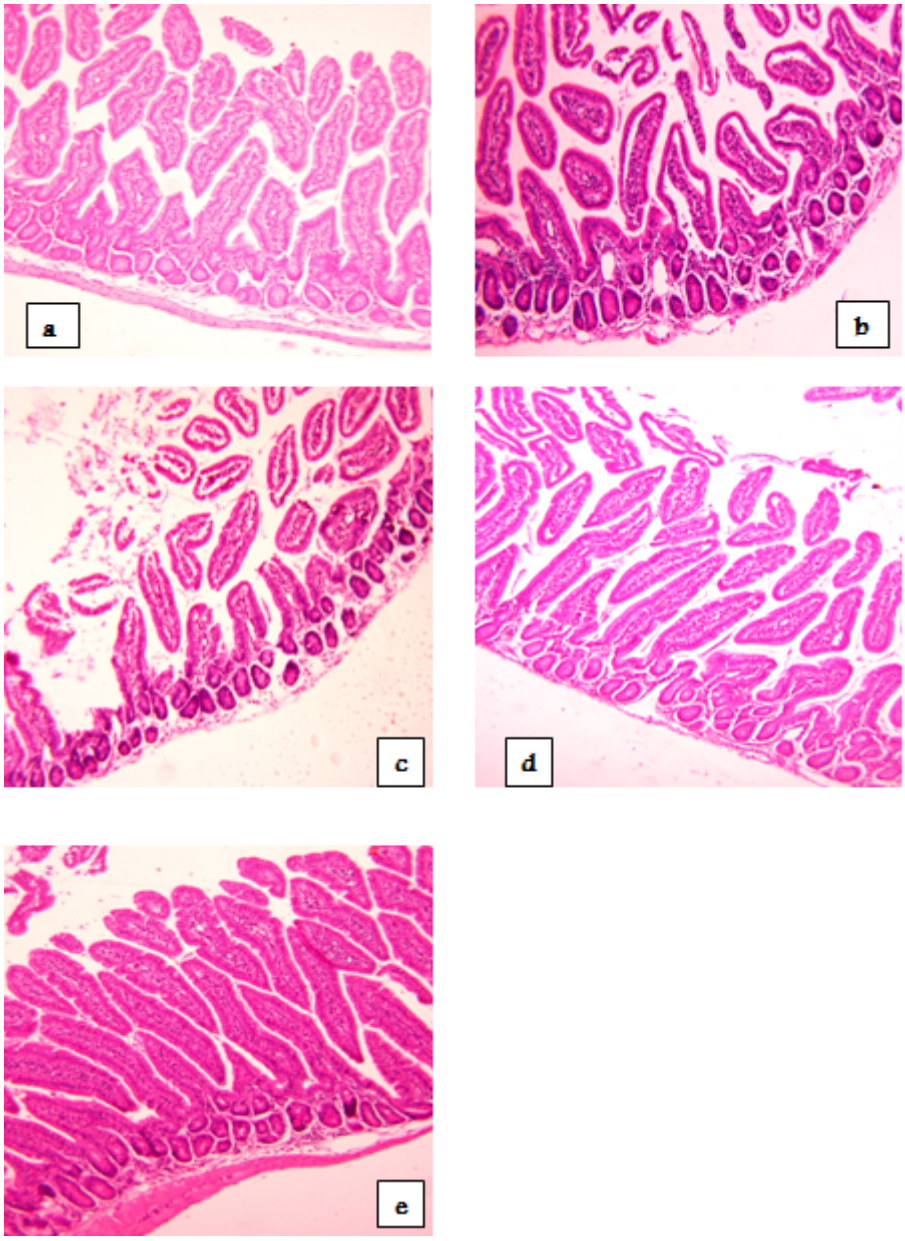

Figure 5

Microscopic observation of jejunal sections. Photomicrographs are representative jejunum samples from mice. (A) Control, (B, C) FB1 alone, (D) LP alone, (E) LP+FB1. Magnification 100X. M: mucous.

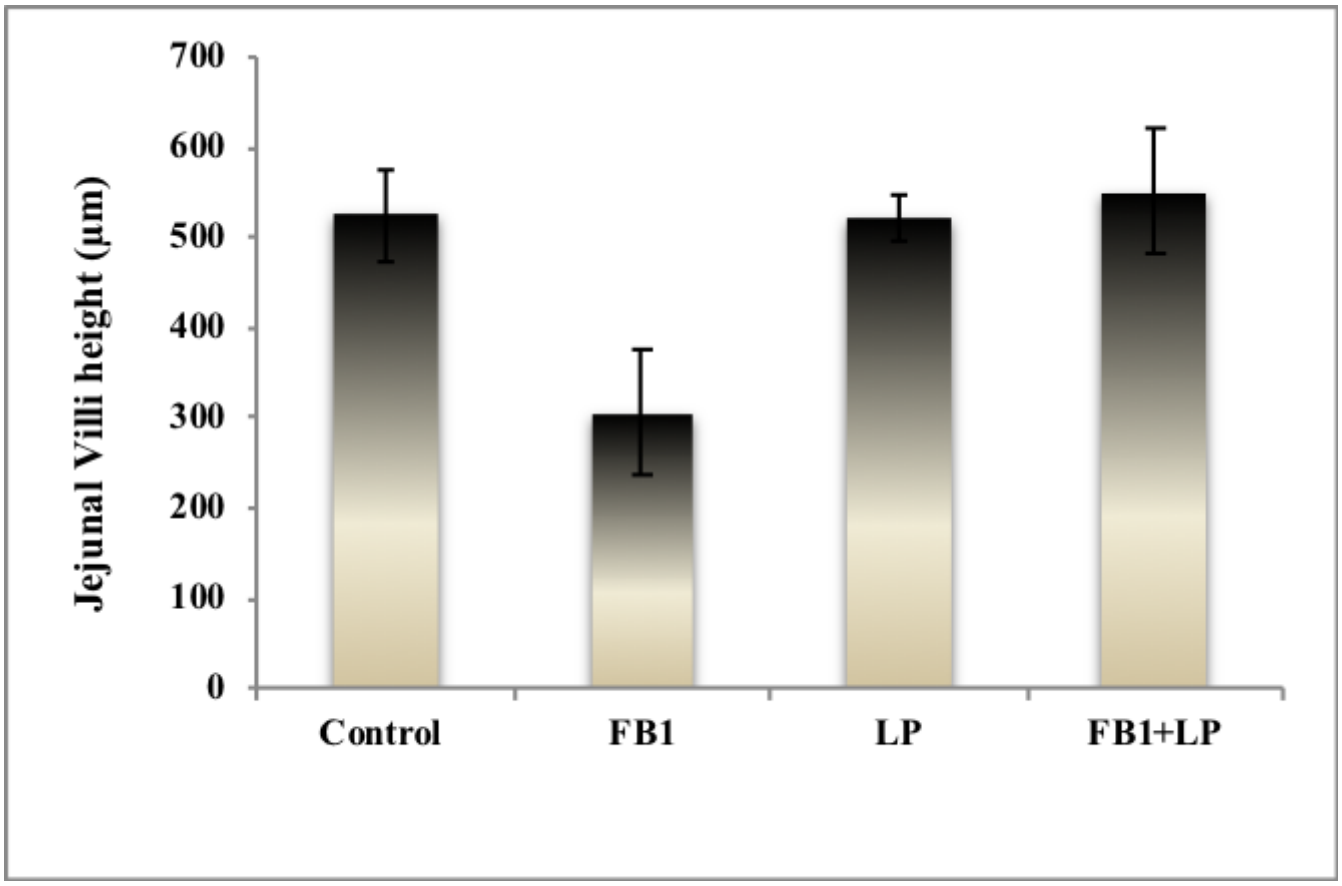

Figure 6

Jejunal villi height length (in µm). *Value of LP+FB1co-treated group are significantly different from FB1 alone treated group ($p < 0.05$).

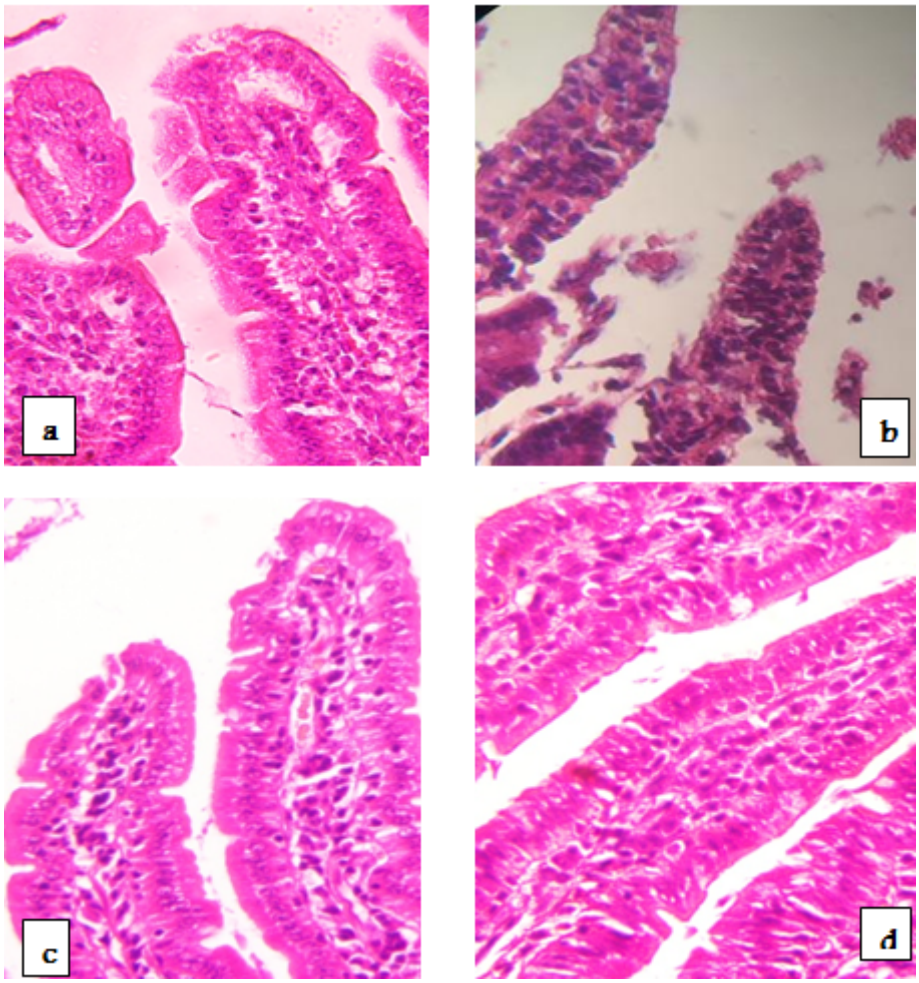

Figure 7

Microscopic observation of jejunal villi of (A) control, (B) FB1 B1, (C) LP alone, and (D) LP+FB1 mice. All samples stained with hematoxylin and eosin (Magnification 400X).