Lactobacillus rhamnosus GG Inhibits BID Dependent-Apoptosis in Human Hepatocellular Carcinoma Cells Exposed to Patulin

Bernice D. Karlton-Senaye[1], Rishipal Bansode[1], Priscilla Randolph[1] & Leonard L. Williams[1]

[1]Center for Excellence in Post-Harvest Technologies, North Carolina Agricultural and Technical State University, North Carolina Research Campus, Kannapolis, North Carolina, United States

Correspondence: Bernice D. Karlton-Senaye, Center for Excellence in Post Harvest Technologies, The North Carolina Research Campus, 500 Laureate Way, Kannapolis, NC 28081, USA. Tel: 704-250-5700. E-mail: bekarlton@gmail.com

Abstract

Patulin, a mycotoxin, which is a major contaminant in apple juices, has contributed immensely to the occurrence of liver diseases. Consumption of apple juice could over long period of time become harmful to the health of individuals with pre-existing liver disease. Probiotics are known for their role in patulin removal from aqueous media. In this study, we investigated the effects of a probiotic microorganism on patulin toxicity in hepatocellular carcinoma (HepG2) cells and established the protective effect of *Lactobacillus rhamnosus* (LGG) as mediated by induction of BH3-interacting domain antagonist (BID) in response to patulin toxicity. After 24 hours of patulin exposure followed by 24 hours of treatment with *Lactobacillus rhamnosus*, cells proliferation decreased with increasing patulin exposure in samples without LGG pre-treatment, whereas with increasing concentration of patulin, cells were relatively rescued in LGG treated samples. It was further observed that pre-treatment of LGG with polysaccharide gums led to a decline in cell proliferation with increasing patulin exposure. Compare to the control, the expression of p53 upregulated moderator of apoptosis (PUMA) increased slightly by 7 % at $10\,\mu M$ patulin exposure in treatment and decreased by 30% in untreated cell. However, the expression of BID decreased by 26% in treatment compared to the control. We further established that the protective effect of *Lactobacillus rhamnosus* was mediated by the inhibition of BID. Our findings suggest that *Lactobacillus rhamnosus* GG could potentially function as a therapeutic agent to reverse the damaging effect of patulin on the liver of individuals with pre-existing liver disease.

Keywords: BID, gums, HepG2, *Lactobacillus rhamnosus* GG, Patulin, Probiotics

1. Introduction

Patulin is a mycotoxin that is secreted by certain species of Penicillium, Aspergillus and Byssochylamys molds that grow in various foods including fruit, grains, and cheese. Patulin is capable of causing damage to both human and animal organs including liver, kidney, intestine and the immune system (Ayed-Boussema et al., 2013). As a result, patulin has been classified as group-3 carcinogen (International Agency for Research on Cancer [IARC], 1987). Patulin has also been found to be teratogenic, carcinogenic, genotoxic, and mutagenic causing DNA damage in certain cases (Ciegler, Becwith, & Jackson, 1976 Osswald, Komitowski, & Winter, 1978, Zhou, Jiang, Geng, Cao, & Zhong, 2009). Previous studies have shown that patulin could induce oxidative DNA damage in many organ areas including liver, kidney, brain, and urinary bladder (de Melo et al., 2012). Patulin has also been found to be genotoxic and mutagenic in Chinese hamster lung fibroblast V79 cells, and hepatoma cells HepG2 (Ayed-Boussema et al., 2013).

Lactobacillus, Bifidobacterium, Escherichia, Enterococcus, Bacillus, Streptococcus, and some fungal Saccharomyces strains have been known as probiotics (Fooladi, Hosseini, Nourani, Khani, & Alavian, 2013). Probiotics, according to WHO are "live microorganisms which when administered in adequate amounts give a health benefit on the host" (WHO, 2003). *Lactobacillus rhamnosus* GG belong to Lactobacillus species considered as probiotics with numerous health benefits. Several studies tested the effects of various strains of probiotics for treating or preventing hepatic disease, inflammatory bowel disease, cancer, helicobacter pylori infection, diarrhoea, vaginosis, allergy, lactose intolerance, high cholesterol levels, colitis, modulation of immune system, and several other abnormalities (Wager, Champagne, Buckley, Raymond & Green-Johnson,

2009; de Vrese, Kristen, Rautenberg, Laue, & Schrezenmeir, 2011; Ejtahed et al. 2011; Khani et al., 2011). It has been confirmed that the consumption of 10^8 to 10^{11} CFU of probiotics per day can impact the healthy effects of the probiotics (Khani et al., 2011), which could be obtained through consumption of a one hundred gram of dairy products or other available commercial formulation containing probiotics (Khani et al., 2011).

BCL-2 family proteins, known for activities such as pro- or anti-apoptotic activities, have been studied intensively for the past decade due to their role in the control of apoptosis, cellular responses, tumorigenesis and to anti-cancer remedy (Youle & Strasser, 2008). The BCL-2 family of proteins comprise of the following three groups the pro-survival proteins (A1/BFL1, BCL-2, BCL-W, BCL-XL, MCL-1), the multi-BH domain pro-apoptotic proteins (BAK, BAX, BOK) and the pro-apoptotic BCL-2 homology 3 (BH3)-only proteins (BAD, BID, BIM, BMF, NOXA, DP5, BLK, and PUMA (Gurzov & Eizirik, 2011). The p53 tumor suppressor could be triggered by exposing cells to stresses leading to induction of apoptosis or cell growth arrest. Exposure of patulin to HepG2 cells could present a stressful condition to already damaged HepG2 cells. BH3 proteins such as BID that prompt direct or indirect activation of other protein senses such stresses (Shamas-Din, Kale, Leber, & Andrews, 2013). Nonetheless, the ability of p53 to remove excess, damaged cells by apoptosis are vital for the regulation of cell proliferation (Haupt, Berger, Goldberg, & Haupt, 2003).

Meanwhile, apple juices, apples, pears, flour, apricots, grape products and malt feed are major foods that promote the occurrence of patulin (Harwig, Chen, Kennedy, & Scott, 973, Ayed-Boussema et al., 2011). Due to processing conditions of most food, patulin appears to cause less safety concerns, with the exception of apple juice (Fritz, 1981). For instance, alcoholic fruits drinks are found to be free of patulin due to fermentation, which destroy patulin in such beverages. Although FDA has regulated levels in apple juice to a max limit 50 µg/kg over the year, patulin is only moderately reduced during pasteurization of apple juice. This is evident as apple juice and cider samples collected in selected stores in Michigan were found to have more than 50 µg/liter patulin concentrations (Harris, Bobe & Bourquin, 2009), thus causing some level of concern for frequent consumption of apples juice specifically by individuals with pre-existing liver disease and other vulnerable groups such as children and the elderly (IARC 1986, WHO, 1990).

However, a number of studies were performed to determine the effects of probiotic on aflatoxin cytoxicity in hepatocellular carcinoma and liver dysfunction. Administration of *Lactobacillus rhamnosus* LC705 in combination with *Propionibacterium freudenreichii subsp. shermanii* led to lower AFB-N7 during a five-week study (El-Nezami et al., 2006). Administration of probiotic homogenate significantly reduced ODC mRNA and activity as well as polyamine content and neoplastic proliferation in HGC-27 cancer cell line (Russo et al., 2007). Although some studies have been performed to demonstrate the positive effect of probiotics on other mycotoxins on selected cancers cell lines very limited studies have been conducted on the effect of probiotic microorganisms on patulin-induced toxicity in HepG2 cell line. We therefore hypothesized that *Lactobacillus rhamnosus* could inhibit patulin-induced toxicity of human hepatoma HepG2 cells and induce PUMA and BID mediated cell damage. The aims of this study were to assess the effect of *Lactobacillus rhamnosus* GG on inhibition of patulin induced toxicity in HepG2 cells and to evaluate the protective effect of *Lactobacillus rhamnosus* GG on pro-apoptotic induction of PUMA and Bid.

2. Materials and Methods

2.1 Materials

The human hepatoma HepG2 cells were obtained from the cell collection at CEPHT (NCAT-CEPHT, Kannapolis, USA). Eagles minimal essential medium (EMEM), Fetal Bovine Serum (FBS) and XTT assay kit (Manassas, VA, USA) were purchased from ATCC. Patulin were purchased from Sigma Aldrich St. Louis, MO, USA. Antibodies specific for total PUMA, BID and β-actin and were purchased from Cell Signaling Technology (Beverly, MA, USA). The following polysaccharide gums namely: inulin (IN), guar (GU), pectin-carrageenan (PC), carrageenan-maltodextrin (CM), locust bean (LB) and guar-locust bean-carrageenan (GLC), were kindly supplied by TIC Gums Incorporates, GA, USA. All reagents used in this study were of analytical reagent grade.

2.2 Cell Culture Conditions

The human hepatoma HepG2 cells were obtained from the cell collection at CEPHT (NCAT-CEPHT, Kannapolis, USA) and were maintained in culture in 75-cm^3 polystyrene flasks (Corning, Pittsburgh, PA, USA) with Eagles minimal essential medium (EMEM). Cells were cultured in EMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, in monolayer culture, and incubated at 37◦C in a humidified atmosphere containing 5% CO_2. At confluence, the cells were harvested by means of trypsinization and used for testing.

2.3 Preparation of Medium and Gums

To determine whether polysaccharide gums could increase the protective effect of *Lactobacillus rhamnosus* GG,, gums were prepared using the method described by Karlton-Senaye et al., 2015, with some slight modifications. Each of the following gums including inulin (IN) guar (GU), carrageenan-maltodextrin (CM), pectin-carrageenan (PC), locust bean (LB) and guar-locust bean-carrageenan (GLC), (TIC Gums Incorporates, GA, USA) at 37 ℃ was gradually dissolved into the 200 mL batches of Lactobacilli deMAN Rogosa and Sharpe (MRS) broth at 0.5% (w/v), pasteurized at 110 ℃ for 15 min and cooled to 50 ℃. Before use, MRS broth were further distributed into batches of 5 mL and inoculated with single pure colony of active culture. MRS without gum served as a negative control.

2.4 Preparation of Lactobacillus rhamnosus GG

Active single pure colonies culture of *Lactobacillus rhamnosus* GG was inoculated into *Lactobacillus* MRS broth containing 0.5% of each of the following polysaccharide gums: inulin (IN) guar (GU), pectin-carrageenan (PC), locust bean (LB) and guar-locust bean-carrageenan and incubated at 37◦C over night to reach the log phase with the density determined as 0.5 at 600 nm. LGG was then prepared according to the method by Russo et al., 2007. LGG was precipitated (1,000 g for 15 min at room temperature) from MRS broth with or without gums and washed twice with phosphate-buffered saline (PBS), pH 7.4 at 800 g for 5 min at room temperature. The content of bacterial cells was released by homogenization on ice at 50 watts for 1 min at 30-s intervals until the cells were disrupted. Prepared sonicated bacteria was then suspended in PBS (pH 7.4) reaching a concentration of 10^8 -10^9 cells/ml. Finally, the preparation was centrifuged (1,000 g, 30 min at 4°C), and the supernatant (ranged from 1.05-1.07 at 600 nM) collected was stored at -80◦C for use the next day (Russo et al., 2007).

2.5 Treatment with Lactobacillus rhamnosus GG

In order to investigating the effects of polysaccharide gums and LGG on cell proliferation, HepG2 cells were seeded in 24 well plates (10^5 cells/well) in EMEM containing 10% Fetal Bovine Serum and allowed for attachment. After 24 h, HepG2 cell were exposed to patulin at 0, 1.0, 2.5, 5.0, 7.5 and 10.0 μM. HepG2 cells were allowed to grow for 24 h and then the medium removed and a fresh media was added. In order to determine effective dose level, LGG was administered at 0, 25, 50, 75 and 100 μL/well. After which 100 uL dosage was selected to be the effective dose for maintenance of cell viability at increasing level of patulin. Subsequently, one hundred microliter (100 μL) per one milliliter of LGG was added to cells and allowed to grow for 24 h and then processed for the analyses. Each experiment included an untreated control (negative control) and a control (positive control) with the equivalent concentration of PBS as added to LGG. Duplicate cultures were set up for each LGG concentration and for control. Each experiment was repeated at least three times. Cell proliferation was determined using XTT assay (ATCC, VA, USA).

2.6 Assessment of Cell Proliferation by XTT Assay

HepG2 cells were seeded in 24 well plates (10^5 cells/well) in EMEM containing 10% Fetal Bovine Serum for 24 h to ensure cell adherence. Cells were then exposed to varying levels of patulin at 0, 1.0, 2.5, 5.0, 7.5 and 10.0 μM for 24h. The media was aspirated after 24 h of exposure and then cell were treated with LGG at 1: 10 (v/v) media. The media was removed and cells were washed with PBS. One milliliter (1mL) of EMEM containing 10% FBS was added to each cell after which 20 μL of XTT assay (ATCC, Manassas, VA, USA) was added to each cell and incubated at 37 °C for 30 min in 5% CO_2 to determine proliferative response. Metabolically viable cells were monitored by spectrophotometer and percentage cell viability determined.

2.7 Total Protein Normalization and Western Blot

Cells were washed twice, and lysed with trypsin. The cell lysate was then placed on ice for 5min followed by total protein normalization using Pierce BCA Protein Assay (Rockford, IL, USA) according to manufactures instruction. One hundred (100 μL) of laemmli sample/DTT solution (900 μl of 4x laemmli sample buffer to 100 μl of DTT) to each well and were allowed to sit for 2 minutes. Samples were allowed to boil for 5 minutes. Fifteen microliters (15 μL) of sample were loaded onto 12% gel and run on 60 volts and then semi-dry transferred onto nitrocellulose paper. The Nitrocellulose paper were washed with saline buffer containing 0.1% Tween (TBST) three times for 15 mins at 5 min interval and blot in 5 % milk solution by 10ml TBST and allowed to rock for 1 hour at room temperature. Nitrocellulose paper were washed with TBST three times and 5% BSA in TBST along with 10 μl primary antibody were added at 1:1000 and incubated at 4 ℃ overnight. Five microliters (5 μl) secondary antibody (1:3000) were added to 10 ml of TBST containing 5% milk and allowed to rock for one and a half hour at room temperature. Nitrocellulose paper were washed three times with TBST and then developed with SuperSignal West Fento Maximum Sensitivity Substrate (Thermo Scientific, Rockford, IL, USA) and photographed using Fotodyne

CCD camera (Fotodyne INC, Wisconsin, USA). The intensity of PUMA and BID were normalized with the intensity of the housekeeping protein ß-actin, which also served as the control and analysed using Quantity One 1-D Analysis Software (BioRad Laboratory Inc, USA).

3. Results

3.1 Determination of Patulin Cytoxicity in HepG2 Cells

The cytotoxic effect of patulin on HepG2 cells after 24 h incubation as measured by the tetrazolium dye-based XTT assay is shown in Figure 1. The exposure of HepG2 cells to varying levels of patulin (0, 1.0, 2.5, 5.0, 7.5 and 10.0 μM) resulted in increasing cell death in relation to the doses administered. The IC_{50} (concentration that induces 50% of cell mortality) value was determined at 2.5 μM (Data not shown). The IC_{50} was considered in the choice for patulin dosage for subsequent experiments.

3.2 Determination of Concentration of LGG on Patulin-Induced Cytotoxicity in HepG2 cells

Figure 1. shows the effect of different concentration of LGG on patulin cytoxicity at varying concentrations. To determine the most effective concentration of LGG that could inhibit patulin toxicity, varying concentrations of (25, 50, 75 and 100 μL) LGG were used to treat HepG2 cells that were exposed to patulin at 0, 1.0, 2.5, 5.0, 7.5 and 10.0 μM. The results showed that the effect of LGG on cell proliferation occurred in dose dependent manner. Relatively higher concentration of cells was rescued at 100 μL of LGG resulting in lower cytoxicity at increasing patulin concentration (Fig. 1). The least effective dose of LGG homogenate on patulin cytoxicity was observed at 25 μL with lowest concentration of cells surviving patulin cytoxicity. It was also observed that at 100 μL of LGG dosage, cell proliferation remained relatively stable within a range of 105.8% to 105.0%, whereas lower LGG pre-treatment resulted in cell death at increasing patulin concentration.
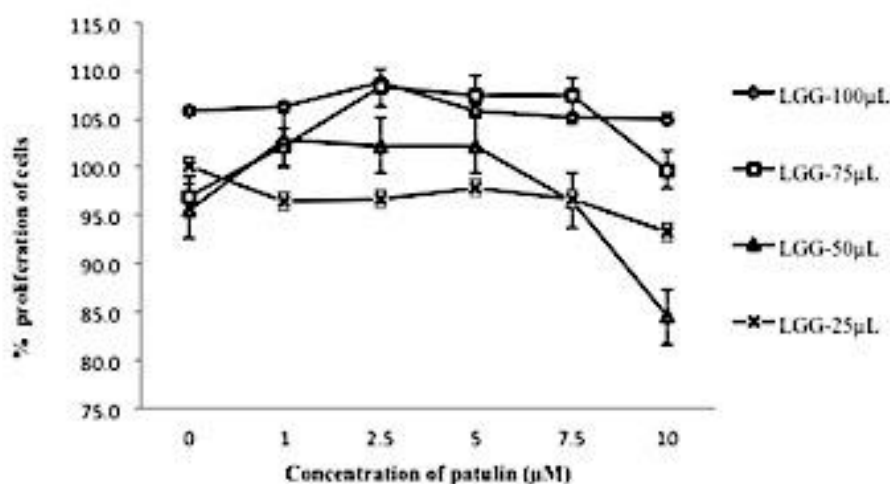

Figure 1. Determining the effective dose of different *Lactobacillus rhamnosus* GG treatment against patulin induced cytotoxicity in HepG2 cells

Note. HepG2 cells were seeded in 6 well plates at (2 x10⁵ cells/well) and treated with 0, 1.0, 2.5, 5.0, 7.5, and 10.0 μM of patulin for 24h followed by exposure with varying amount of *Lactobacillus rhamnosus* GG (25, 50, 75 and 100 μL/mL) for 24h. The XTT assay was added to each well after the 48-hour treatment period and incubated as previously described. Cell proliferation was determined by a spectrophotometer.

Figure 2 showed the comparison between the effects of *L. rhamnosus* GG that was pre-treated with 0.5% of different polysaccharide gums on proliferation of HepG2 cells treated with patulin to non-*L. rhamnosus* GG treated samples. Results showed that the treatment of patulin-induced HepG2 cells (10 μM) with *L. rhamnosus* only led to a slight decline from 115.9% to 108.4% at 10 μM. However, pre-treatments of LGG with PC and LB led to increased cell proliferation compared to the control (CT) without treatment. Samples that were exposed to patulin followed by locust bean (LB) pre-treated *L. rhamnosus* led to increased cell proliferation from 77.5 % to 74.7% at 5 μM of patulin exposure, after which cell proliferation declined to 68.5% at 10 μM. Similarly, sample that were pre-treated with IN also showed identical trend but with a lesser cell proliferation effect. Sample pre-treated with GU, CM, and GLC showed a continuous decline in cell proliferation starting at 2.5 μM.

Compared to control all samples treated with gums resulted in decreased cell proliferation with GU treatment causing the highest cell death with increasing dose exposure to patulin. As a result, LGG was subsequently used without pre-treatment with gums for further experiments.

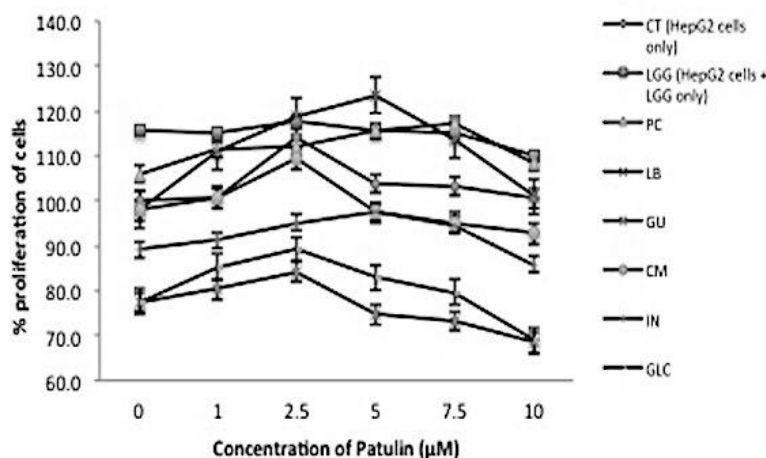*3.3 Determination LGG Pre-treated with Polysaccharide Gums on Patulin Cytotoxicity in HepG2 Cells*

Figure 2. Synergism of gums and *Lactobacillus rhamnosus* GG on proliferation of HepG2 cells treated with patulin

Note. HepG2 cells were seeded in 24 well plates at (10^5 cells/well) and treated with patulin (0-10 μM) for 24 h followed by treatment with *Lactobacillus rhamnosus* GG for 24h at the indicated concentrations. The XTT assay was added to each well after the 48-hour treatment period and incubated as described previously. Cell proliferation of control   (CT, HepG2 cells only) and treatments LGG (HepG2 cells exposed to LGG only); PC (HepG2 cells exposed to LGG that was pre-treated with pectin-carrageenan); LB (HepG2 cells exposed to LGG that was pre-treated with locust bean); GU (HepG2 cells exposed to LGG that was pre-treated with guar); CM (HepG2 cells exposed to LGG pre-treated with carrageenan-maltodextrin); IN (HepG2 cells exposed to LGG that was pre-treated with inulin); and GLC (HepG2 cells exposed to LGG that was pre-treated with guar-locust bean-carrageenan) were determined spectrophotometrically.

3.3 Effect of Lactobacillus rhamnosus on Patulin Cytoxicity

To determine whether LGG could inhibit cell death caused by patulin and enhance proliferation, human liver hepatoma cells were cultured with 0, 1.0, 2.5, 5.0,7.5 and 10.0 μM patulin for 24 h followed by treatment with LGG at 100 μL/mL after which proliferation was determined at 24 hours. The result of LGG inhibition of patulin-induced cytotoxicity is observed in Figure 3. LGG was found to rescue cells death caused by patulin toxicity. It was also observed that patulin induced-cell death occurred in dose dependent manner. Increasing concentration of patulin led to a study decline in cell viability from 79.40% at 0 μM patulin exposure to 25.90 % at 10 μM of HepG2 cells that were not treated with LGG due to increasing toxicity. Inclusion of LGG, on the other hand, resulted in cell rescue and arrest of cytotoxicity with only a slight decline from 160.40 % at 0 μM to 142.77 % at 10 μM of patulin exposure. Cell proliferation increased almost by two-fold at each concentration of patulin in treated sample compared to the control. However, at 10 μM of patulin concentration cell viability increased by over five times from 142.70 % in treatment compare to 25.90 % in control without LGG treatment.
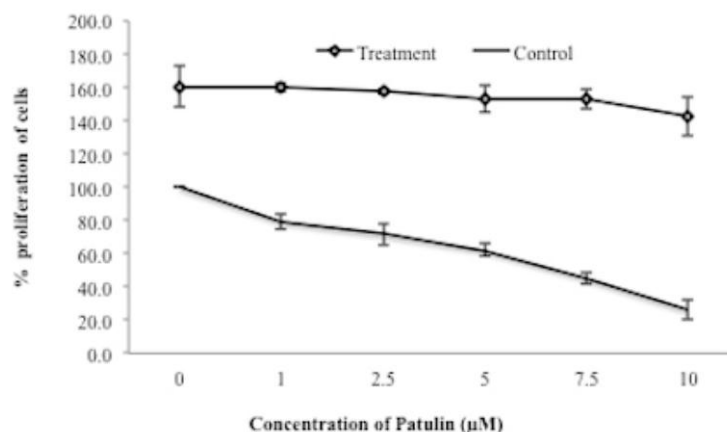
Figure 3. Percentage effect of *Lactobacillus rhamnosus* on proliferation of HepG2 cells treated with patulin

Note. HepG2 cells were seeded in 6 well plates at (2 x10^5 cells/well) and treated with 0, 1.0, 2.5, 5.0, 7.5, and 10.0 μM of patulin for 24h followed by treatment with *Lactobacillus rhamnosus* GG (100 μL/mL) for 24h. The XTT assay was added to each well after the 48-hour treatment period and incubated as previously described. The percentage proliferation of control (CT, HepG2 with or without patulin, without LGG treatment) and treatments (HepG2 cells with or without patulin, but with LGG treatment) were determined spectrophotometrically.

3.4 Effect of Lactobacillus rhamnosus on Cell Damage Induced by PUMA and BID

The ability of patulin to inhibit the proliferation of liver heptoma cells couple with the ability of LGG to rescue patulin-induced cytotoxicity of liver heptoma cells led to further investigation into expression of proapoptotic BCL2 family members PUMA and BID as shown in Figures 4A,B,C. Cells were treated with 0-10 μM patulin for 24 h followed by treatment with LGG. Protein expression profile revealed an increased induction of PUMA and a decrease induction of BID in treatments compared to control as shown in Figures 4A and 4B.

The expression of PUMA increased by 20% in the treatments compared to the control at 10μM patulin exposure (Fig. 4A). Similarly, a slight increase in PUMA expression was observed in cells that received both patulin and LGG treatment (7%) compared to cell exposed to patulin only with no LGG treatment. However, the expression of PUMA in cell, which received no exposure to both patulin and LGG was higher (100%) than that with both treatments (70%), thus showing some protective effect of LGG to patulin exposed HepG2 cells (Fig. 4A).

A reverse observation was made in the case of expression of BID by LGG (Figure 4B.). The induction of BID decreased from 100% in untreated to 86.22% in LGG treated cells. Similarly, the expression of BID also decreased from 115.73% in "patulin only" treated cells to 89.56% in "patulin and LGG" treated cells (Fig. 4B.) at 10 μM of patulin exposure. BID was equally expressed in treated and untreated cell at 7.5 μM of patulin treatment (Data not shown). Expression of PUMA and BID are consistent with apoptotic genes associated with cell damage. β-actin, a housekeeping protein, which served as common loading control showed no difference in expression related to cytotoxicity (Fig. 4C)
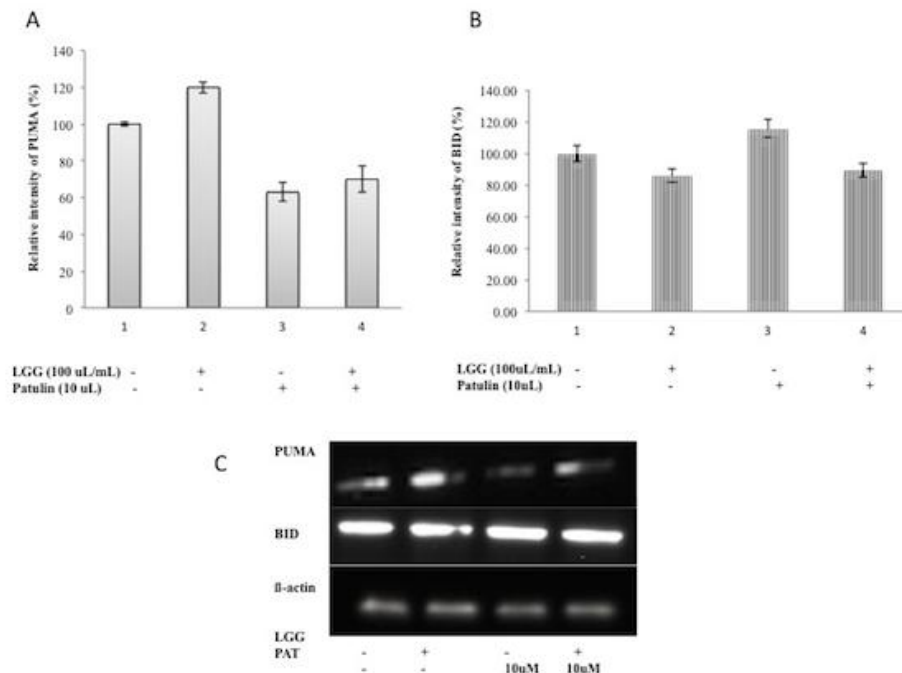
Figure 4. Induction of PUMA and BID in hepatocellular carcinoma cells (HepG2) after treatment over time with LGG

Note. HepG2 cells were exposed to 10 uM patulin for 24 h followed by treatment with LGG (100 μL/mL). Proteins (15 μg) were separate using western blotting. β-actin was used as a loading control. The intensity values of the protein bands were normalized against the housekeeping protein ß-actin, which also served as the control. The treatment were: 1: HepG2 cells not exposed to patulin nor treated with LGG; 2: HepG2 cells treated with LGG but not patulin; 3: HepG2 cells exposed to patulin but not LGG; and 4: HepG2 cells exposed to patulin following treatment with LGG.

4. Discussions

Patulin is a toxic contaminant of fruits with subsequent immunotoxic, genotoxic, embryotoxic, neurotoxic and carcinogenic effect due to consumption over a period time (Ayed-Boussema et al., 2011).   Although the World Health Organization set a limit for patulin concentration in food, studies have indicated that patulin is quiet stable in apple juice despite the processing steps. Best practices such as good agricultural practices (GAP) and good manufacturing practices (GMP) throughout the processing of the juice are crucial in eliminating this mycotoxin (Sant'Ana, Rosenthal, & de Massaguer, 2008). As a result, some commercialized products contain patulin that has exceeded the regulatory levels (Baert et al., 2006; González-Osnaya, Soriano, Moltó, & Mañes, 2007; Harris, Bobe, & Leslie, 2009) that could cause cell damage due to cumulative effect. Therefore, using probiotics, which have numerous health benefits in addition to inhibiting patulin-induced toxicity in HepG2 cells, could further promote the role of probiotics as therapeutic agents for an improved liver function and metabolisms against patulin toxicity.

4.1 Dose Determination for LGG Treatment

Cell survival induced by probiotic LGG showed a dose dependent trend. This could be attributed to increase in enzyme concentration with increase dose of LGG homogenate resulting in increased removal of patulin leading to protection of HepG2 cells by probiotic LGG). This trend was expected as probiotics have been used to either remove or reduce bioavailability of mycotoxins from aqueous medium. The increased in cell rescue could also be attributed to the repair of cell which prevented further damage and cell death (Fuchs et al., 2008).

4.2 Cell Proliferation and effect of Lactobacillus rhamnosus GG

Increasing patulin concentration was expected to cause increase in cell death resulting from increasing toxicity, which was observed in both the treatments and the control. Similarly, the increased in cell proliferation in the treatment could be attributed to possible removal of patulin and repair of the cells from cell damage induced by

patulin exposure. The protective effect of LGG resulting in cells rescue was expected as Zoghi et al.in their study, reported that probiotic bacteria is capable of binding patulin and other mycotoxins in vitro by metabolic conversion of toxins through bacterial enzymatic action (El-Nezami et al., 2006; Zoghi et al., 2014). El-Nezami et al (2000) have demonstrated that lactic acid bacteria, most of which are probiotic, are also capable of detoxifying aflatoxins B, a well know potent carcinogen, which was a major contributor of liver cancer in central Africa and China (El-Nezami et al., 2000, IARC, 2002). Binding of patulin by LGG thus led to relative rescue of the liver cells, leading to inhibitory effect of patulin induced toxicity in the presence of increasing levels of patulin.

4.3 Cell Damage Induced by PUMA and BID

PUMA (p53 upregulated modulator of apoptosis) and BID, both of which are pro-apoptotic BCL2 proteins were induced by patulin and LGG in the HepG2 hepatocellular carcinoma cell line. The induction of PUMA and BID by LGG could be due to the damage effect caused by the exposure of cells to patulin or the increase in cells proliferation caused by LGG leading to cytotoxicity by the HepG2 cells themselves, which correlates with our finding of LGG producing increasing cell proliferation (Figure 3). Patulin is known to induce DNA damage leading to p53 mediated cell cycle arrest (Saxena et al., 2009; de Melo et al., 2012). It has been proven that p53 induces apoptosis by converting a few target genes, including certain BCL-2 family members, and also by directly targeting mitochondria. Gross Atan (2006) reported that BID and P53 are known to play balance between life and death. Therefore, it could be stated that PUMA and BID interconnect following DNA damage. Numerous proteins have been identified that can enhance the interaction of p53 with the promoters of apoptotic genes. Thus, the slight increased expression of PUMA protein due to exposure to patulin and LGG could lead to DNA damage to HepG2 cells to specific response and pathways in relation to apoptosis. A decrease in the expression of BID following treatment of the patulin-exposed cells with LGG could be due to reduction in cell death and repair of DNA damage caused by the LGG treatment (Gross Atan, 2006).

In conclusion, for this current study, we evaluated the protective effect of *Lactobacillus rhamnosus* GG from patulin-induced apoptosis in hepatocellular carcinoma cells by induction of PUMA and BID. The results showed that the treatment of HepG2 cells with LGG inhibited patulin-induced toxicity. Additionally, LGG increased cell death of damaged cell through the induction of BID. The inhibition of patulin-induced toxicity of hepatocellular carcinoma cells by LGG through the induction of BID could have a potential in the application for therapeutic formulations to reduce toxic effects of patulin in individuals with pre-existing liver disease through an enhanced treatment. However, further research is required to investigate the mechanisms involved in this phenomenon.

Acknowledgment

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