Hepatotoxicity and genotoxicity of patulin in mice, and its modulation by green tea polyphenols administration

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A B S T R A C T

Patulin (PAT) is a mycotoxin produced by certain species of Penicillium, Aspergillus, and Byssochlamys. Previous studies demonstrated its cytotoxic, genotoxic, and mutagenic effects in different cell lines. However, there is little information available concerning its toxic behavior in vivo. In the present study, we investigated PAT-induced hepatotoxicity and genotoxicity in mice. We also investigated the antioxidant and anti-genotoxicity efficiency of green tea polyphenols (GTP) against PAT-induced toxicity. We found that PAT-treatment induced serum alanine transaminase (ALT) and aspartate transaminase (AST) activities significantly. PAT-induced lipid peroxidation was confirmed with the elevation of thiobarbituric acid-reactive substances (TBARS). Moreover, the increasing of reactive oxygen species (ROS) and decreasing of GSH level implied its oxidative damage mechanism. In bone marrow cell, PAT was found to induce micronucleus and chromosomal aberration formation. In addition, our result suggested that GTP administration has dose-dependent antioxidative and antigenotoxic effect in against PAT-induced hepatotoxicity and genotoxicity.

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1. Introduction

Patulin (PAT) is a mycotoxin produced by genera Penicillium, Aspergillus and Byssochlamys (Mahfoud et al., 2002; Sanzani et al., 2012). PAT has been isolated from a number of fruits and vegetables, mainly apples and apple products (Moake et al., 2005). Previous studies demonstrated PAT contamination poses a serious health risk to consumers, various forms of acute and chronic effects of PAT have been characterized (Moake et al., 2005; Sant’Ana et al., 2008). European Union has regulated the maximum permitted levels of PAT in apple juices (50 ppb), purees (25 ppb) and, above all, baby foods (10 ppb) (Sanzani et al., 2012). World Health Organization (WHO) and US Food and Drug Administration (FDA) also limit patulin to 50 ppb as the recommended limit in apple juice (Puel et al., 2010; Van Egmond, 1989).

PAT has obvious electrophilic reactivity, leading to the formation of covalent adducts when it interacts with electrophilic chemicals. The main cellular target of PAT is cysteine-containing tripeptide glutathione (GSH). Indeed, PAT-intoxication induced a quick depletion of GSH in cultured cell (Yang et al., 2011) and in mice (de Melo et al., 2012; Pfeiffer et al., 2005). In addition, PAT also reacts with free cysteine, or cysteine-, lysine-, histidine-containing proteins, and most of the toxic effects of PAT-treatment are considered as the consequences of this adduct-forming activity (Papp et al., 2012). On the other hand, several studies implicated PAT-mediated toxicity via oxidative damage pathway. PAT was proved to increase reactive oxygen species (ROS), 8-hydroxydeoxyguanosine (8-OHdG) and thiobarbituric acid-reactive substances (TBARS) contents (Ayed-Boussema et al., 2013; Ferrer et al., 2009).

Numerous studies showed that phytochemicals protect against the toxic effects induced by environmental pollutants (Celik et al., 2013). Epidemiological studies indicated the consumption of these phytochemicals is associated with health benefits of reducing oxidative related damages (Borek, 2004). Green tea polyphenols (GTP) are the most important biological active components of green tea. GTP have significant antioxidant properties and exert protective role in the development of cancer, cardiovascular disease and other pathologies (Dreosti, 1996; Dufresne and Farnworth, 2001;
Fuji et al., 2002; Riegeceker et al., 2013). However, there is no information regarding to the effect of GTP modifying PAT-induced toxic response.

In the present study, we aimed to (1) evaluate the hepatotoxicity of PAT, (2) evaluate the genotoxicity of PAT in bone marrow and (3) investigate the protective effect of GTP at three different doses against damages occurred by PAT. Silymarin has been widely used in clinical practice for the treatment of toxic liver diseases (Lee et al., 2007). Its antioxidant capacity and hepatoprotective effect have been extensively reviewed (Poylak et al., 2013; Vargas-Mendoza et al., 2014). In the current study, silymarin was served as a well-characterized antioxidant control for PAT-induced hepatotoxicity and genotoxicity.

2. Materials and methods

2.1. Chemicals

Patulin (PAT, 4-hydroxy-4H-furo[3,2-C]pyran-2(6H)-one, purity >98.0%, Fig. 1), GTPs (Polyphenol 60 from green tea) and 2′,7′-dichlorofluorescein-diacetate (DCFH-DA) were obtained from Sigma–Aldrich (Shanghai, China). The components of GTPs include epicatechin (8.5%), epigallocatechin (17.7%), epigallocatechin gallate (32.1%), epicatechin gallate (10.7%), gallicolin gallate (3.3%), and catechin gallate (1.4%) (Lee et al., 2008). Diagnostic kits of superoxide dismutase (SOD) and catalase (CAT) activities, serum alanine transaminase (ALT) and aspartate transaminase (AST) activities, GSH and thiobarbituric acid reactive substances (TBARS) content were obtained from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). All other chemicals used were of the highest grade commercially available.

2.2. Animals and treatment

All animal experiments were performed in accordance with the guideline of the Animal Care Committee of Southwest University. All efforts were made to minimize suffering. Male Kunming mice (22 ± 2 g) were purchased from Chongqing Tengxin Biotechnology Co. Animals were maintained under standard conditions of humidity (50%), temperature (25 ± 2 °C) in a 12 h light/12 h dark cycle. They were fed standard rodent chow and had free access to water. After one week’s acclimation, thirty-six mice were randomly divided into six groups with six mice in each group. Mice received daily intraperitoneal (i.p.) injection of high dose (100 mg/kg), medium dose (50 mg/kg) or low dose (25 mg/kg) of GTP for 7 days continuously. PAT (1 mg/kg,i.p.) was injected 1 h after the last GTP administration. GTP was dissolved in physiological saline and PAT was dissolved in physiological saline containing 0.1% DMSO. Solvent control group received a physiological saline containing 0.1% DMSO. The last group was given silymarin (100 mg/kg, i.p.) for 7 days continuously prior to PAT treatment. The mice were grouped as follows:

Group 1: vehicle control, received 0.1% DMSO in saline (2 mL/kg BW/d).
Group 2: received PAT (1 mg/kg).
Group 3: received PAT (1 mg/kg) and GTP (25 mg/kg BW/d).
Group 4: received PAT (1 mg/kg) and GTP (50 mg/kg BW/d).
Group 5: received PAT (1 mg/kg) and GTP (100 mg/kg BW/d).
Group 6: received PAT (1 mg/kg) and silymarin (100 mg/kg BW/d).

After the PAT injection for 24 h, mice were anesthetized with CO2. Blood samples were collected and serum was separated to determine serum ALT and AST activities. Liver were rinsed in ice-cold physiological saline and homogenized in Tris–HCl buffer (0.01 M, pH 7.4) to give a 10% homogenate. Homogenates were centrifuged at 3000 rpm, 4°C for 10 min and supernatant was collected for ROS, TBARS, GSH level and antioxidant enzyme activities. Bone marrow was flushed out for micronucleus and chromosomal aberration tests.

2.3. Assay for serum transaminase activity

Serum from individual mice were separated by centrifugation of blood at 600 g for 15 min and stored at −20 °C until use. AST and ALT activities in the serum were determined spectrophotometrically using test kits (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China), according to the manufacturer’s instructions.

2.4. Determination of ROS level

ROS level was determined using DCFH-DA as fluorescent probe (Pinto et al., 2012). In brief, the assay buffer contained 20 mM Tris–HCl, 130 mM KCl, 5 mM MgCl2, 20 mM NaH2PO4, 30 mM glucose and 5 μM DCFH-DA. The mixture was incubated at 37 °C for 15 min and terminated the reaction by H2O2. The fluorescence was recorded with an excitation wavelength of 488 nm and an emission wavelength of 526 nm (F7000, HITACHI). The average fluorescence of the control group was set to 100%.

2.5. Determination of reduced GSH content

Reduced GSH levels were determined using Ellman’s reagent (Ellman, 1959). Tissue suspension was double diluted and 5% trichloroacetic acid was added to precipitate protein. The supernatant was centrifuged at 10,000 g for 5 min. Ellman’s reagent was added and the absorbance was measured at 412 nm using a UV–Vis spectrophotometer (UV-2450, SHIMADZU). A standard curve was formulated using different concentrations of GSH solution.

2.6. Determination of lipid peroxidation products

Lipid peroxidation was detected by measuring the concentration of TBARS in fluorescence at 532 nm (F7000, HITACHI), using a TBARS detection kit according to the manufacturer’s instructions. The absorbance of was measured at 535 nm. TBARS concentrations of the samples were calculated using the extinction co-efficient of 156,000 M−1 cm−1.

2.7. Determination of antioxidant enzyme activities in liver

SOD and CAT activities were measured with commercial assay kits, according to the previously described method (Liu et al., 2012). SOD activity was determined based on the reaction of SOD and nitrotetrazolium blue chloride (NBT). Briefly, 2.8 ml of reactive mixture (xanthine 0.3 mM, EDTA 0.07 mM, 150 μM NBT, Na2CO3 0.4 mM, bovine albumin 30 mg/30 mL) was added to 0.1 mL sample and 50 μl xanthine oxidase (0.2 M), incubated at 25 °C for 20 min and mixed with 0.1 mL 8 M CuCl2. The absorbance was recorded at 550 nm. CAT activity was determined by measuring the rate of H2O2 decomposition at 240 nm in a reaction mixture containing 10 mM H2O2, 50 mM KH2PO4, and 50 μM of sample. The results were expressed as units of enzyme activities calculated per milligram of protein (U/mg protein).

2.8. Bone marrow micronucleus test

Ends of the femurs were cut off and the bone marrow was flushed out using 0.5 mL of fetal calf serum. Bone marrow cells were centrifuged at 1200 rpm/10 min and the pellet was re-suspended in 0.5% KCl solution and incubated at 37 °C for 25 min. Cells were re-centrifuged at 1200 rpm/10 min and fixed in cold Carnoy’s fixative (acetic acid/methanol, 1:3, v/v) three times. Cell was spread on a slide by pulling one drop of bone marrow suspension behind a cover glass held at a 45°. Slides were then air-dried, fixed in methylal and stained with May-Grunwald/ Gienna and evaluated with a fluorescence microscope (OLYMPUS IX71). For each experimental group, 6 mice were used, and 1000 erythrocytes were observed from each animal to determine micronuclei in polychromatic erythrocyte (MNpCE, %), micronuclei in normochromat erythrocytes (MNCE, %) and polychromatic erythrocyte/polychromatic erythrocyte*normochromat erythrocyte in 1000 cells (PCE/1000 (PCE + NCE)).

2.9. Bone marrow chromosomal aberration test

Fixed bone marrow cells were re-suspended and dropped onto chilled slides, flame-dried and stained the following day in 5% buffered Giemma (pH 6.8). Well spread 100 metaphases per animal (600 metaphases per group) were examined to score the aberrations, as described previously (Savage, 1976). Metaphases with chromatid/chromosome gaps and breaks were recorded and expressed as percent-age of total metaphases per group.

2.10. Statistical analysis

Results were expressed as the means ± S.D. Statistical significance was determined by one-way analysis of variance (ANOVA) using SPSS 18.0 software. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. p < 0.05 was considered to be significant.

Fig. 1. Chemical structure of PAT.
3. Results

3.1. Effect of PAT on serum aminotransferases

PAT-intoxication caused severe hepatotoxicity, as indicated by the significant elevation of serum ALT and AST activities, p < 0.001. However, 25, 50 and 100 mg/mg GTP administration for 7 consecutive days prevented these elevations in a dose-dependent manner, the comparison of mean values of ALT and AST activities in 100 mg/kg GTP group with PAT group showed statistically significant differences p < 0.001, respectively. The effects of GTP on the PAT-induced increase of serum ALT and AST activities were shown in Table 1. Interestingly, we found in our preliminary study that GTP treatment alone has no significant effect on ALT and AST activities (data not shown). Silymarin also significantly reversed the alterations of ALT and AST levels compared with PAT group (p < 0.001 and p < 0.01 respectively).

3.2. Effect of PAT on the levels of hepatic ROS, GSH and TBARS

Levels of ROS, GSH and TBARS in mice liver were described in Table 2. Significant elevation in ROS level was observed in mice received a single dose of PAT (1 mg/kg, i.p.) as compared to the solvent control. GTP administration caused significant reduction in the ROS formation (p < 0.001). GSH plays an important role in hepatic antioxidant defense against ROS mediated oxidative damage. In the current study, GSH level was significantly decreased with PAT-intoxication. Treatment with GTP prevented the decrease of GSH suggesting the protective effect of GTP. Lipid peroxidation is considered to be an important index of oxidative stress related organ pathophysiology. As shown in Table 2, significant increase of TBARS content was observed in PAT-treated group (p < 0.001), while the administration of GTP (50 and 100 mg/kg) significantly decreased TBARS levels (p < 0.01), compared with PAT group. Therefore, GTP showed its antioxidant activities on suppression of PAT-induced oxidative liver damage.

3.3. Effect of PAT on hepatic antioxidant enzyme activities

As shown in Table 3, the activities of SOD and CAT in the liver of PAT-challenged mice were inhibited significantly, compared with solvent control group (p < 0.001, respectively). However, GTP-pre-treated animals showed a dose-dependent improvement of these antioxidant enzymes, silymarin at the dose of 100 mg/kg also could obviously elevate SOD and CAT levels.

3.4. Effect of PAT on bone marrow micronucleus frequencies

PAT-treatment induced a significant increase of micronucleus frequency in mice bone marrow cell. Both of the percentages of micronuclei in polychromatic erythrocytes (MNPCe) and micronuclei in normochromatic erythrocyte (MNNE) in PAT group were elevated with respect to control group, respectively (Fig. 2). However, decrease of MNPCe and MNNE were observed at all three doses of GTP administration, significance were of p < 0.001 at highest dosage GTP-treatment (100 mg/kg). Consistently, statistically significant decline of MNPCe (p < 0.05) and MNNE (p < 0.001) were also observed in silymarin group, compared with PAT group. Furthermore, the cytotoxic potential of PAT was evaluated a ratio of PCE/ (PCE + NCE) among a total of 1000 cells. This ratio decreased significantly in PAT group (p < 0.05) compared with control group, and this declining was significantly prevented (p < 0.05) by GTP (100 kg/mg group) or silymarin treatment.

3.5. Effect of PAT on bone marrow chromosome aberrations

Results given by this assay showed PAT induced chromosome aberrations both in gaps and breaks (Fig. 3). PAT-treatment induced a significant increase in chromosomal gaps and breaks in mice bone marrow cell (p < 0.001 and p < 0.001 respectively). However, GTP treatment (25, 50 and 100 mg/kg) resulted in a dose-dependent decrease of the total chromosomal aberrations. Silymarin-treatment also showed protective role on ameliorate PAT-induced chromosomal aberrations.

4. Discussion

PAT was isolated in 1940s and classified as a mycotoxin in 1960s, however, till today, it still represents a serious health concern to processed-fruit customers (Moake et al., 2005). Therefore, the mechanisms of PAT-induced toxicity are particular interest. In order to understand the adverse effects of PAT, extensive investigations were carried out at cellular level, however, there is not many studies unveiled the mode-of-action of PAT in vivo. In the current study, an attempt was made to investigate patulin-induced hepatotoxicity and genotoxicity in mice. Based on the estimated human exposure levels, the comparable PAT dose in mice would be 1.272 mg/kg/day (Becchi et al., 1981; GC et al., 1998). The LD50 (i.p.) values of PAT on mice was 7.5 mg/kg body weight (McKinley and Carlton, 1980). In the current study, the dosage of 1.0 mg/kg body weight was used throughout. The dosage of 1.0 mg/kg also matches the lowest dosage of previous in vivo study (de Melo et al., 2012). There has no animal death before the sacrifice in our experiment. It is interesting to point out that several studies use oral route for PAT administration for sub-acute toxicity study (GC et al., 1998; Schutze et al., 2010). Oral route is more close to the real exposure scenario since patulin mainly contaminate processed food, but it usually takes longer duration period (usually weeks to months). In the current study, we use the route of i.p. injection for acute experimental model, which has high bioavailability and damages were found with a single injection. Animals in group 3–5 received a single i.p. dose of 25, 50 or 100 mg/kg GTP, and the dosage was selected based on prior studies which shown antioxidant activity (Liu et al., 2008; Xu et al., 2007).

In previous publication, PAT decreased GSH level and GST activity and increased DNA damage in mice liver or liver slices suggested may PAT affects the functions of liver (Pfeiffer et al., 2005). Patulin also depressed protein synthesis in hepatoma tissue culture (Arafat and Musa, 1995), inhibited the activity of hepatic aldolase (Sakthisekaran and Shanmugasundaram, 1990) and decreased the viability of hepatic cells (Zhou et al., 2009). These results also confirmed the liver is the target organ of PAT toxicity. Here, we first showed PAT-induced the elevation of activities of ALT and AST in serum, which are useful biomarkers of liver injury. Since GTP prevented the effect of PAT on serum ALT and AST...
The effect of GTP on hepatic ROS, GSH and TBARS level in PAT-intoxication mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>ROS (% of Control)</th>
<th>GSH (mg/g protein)</th>
<th>TBARS (μmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 12.75</td>
<td>10.82 ± 2.21</td>
<td>1.33 ± 0.12</td>
</tr>
<tr>
<td>PAT</td>
<td>142.28 ± 20.75**</td>
<td>4.43 ± 0.97**</td>
<td>2.33 ± 0.34**</td>
</tr>
<tr>
<td>PAT + GTP (25 mg/kg)</td>
<td>138.73 ± 16.10</td>
<td>6.27 ± 1.27</td>
<td>2.22 ± 0.39</td>
</tr>
<tr>
<td>PAT + GTP (50 mg/kg)</td>
<td>123.55 ± 14.73*</td>
<td>6.67 ± 1.52</td>
<td>1.65 ± 0.39**</td>
</tr>
<tr>
<td>PAT + GTP (100 mg/kg)</td>
<td>96.73 ± 12.51***</td>
<td>8.68 ± 3.23**</td>
<td>1.53 ± 0.30**</td>
</tr>
<tr>
<td>PAT + silymarin (100 mg/kg)</td>
<td>105.48 ± 8.82###</td>
<td>6.2 ± 1.35</td>
<td>1.83 ± 0.61*</td>
</tr>
</tbody>
</table>

Data are presented as the means ± S.D., n = 6.
*** p < 0.001 vs control group.
** p < 0.01 vs PAT group.
* p < 0.05 vs PAT group.

Table 2

The effect of GTP on hepatic antioxidant enzymes in PAT-intoxication mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.78 ± 1.42</td>
<td>5.30 ± 0.55</td>
</tr>
<tr>
<td>PAT</td>
<td>16.70 ± 2.99**</td>
<td>3.51 ± 0.49**</td>
</tr>
<tr>
<td>PAT + GTP (25 mg/kg)</td>
<td>16.57 ± 3.27</td>
<td>4.11 ± 0.52*</td>
</tr>
<tr>
<td>PAT + GTP (50 mg/kg)</td>
<td>23.55 ± 5.61**</td>
<td>4.35 ± 0.38**</td>
</tr>
<tr>
<td>PAT + GTP (100 mg/kg)</td>
<td>23.05 ± 6.29**</td>
<td>5.25 ± 0.26**</td>
</tr>
<tr>
<td>PAT + silymarin (100 mg/kg)</td>
<td>28.95 ± 1.60***</td>
<td>5.57 ± 0.39***</td>
</tr>
</tbody>
</table>

Data are presented as the means ± S.D., n = 6.
*** p < 0.001 vs control group.
** p < 0.01 vs PAT group.
* p < 0.05 vs PAT group.

In PAT-intoxicated mice, the injury of oxidative stress is evidenced by the decreased activity of ALT and AST as well.

Lipid peroxidation has been implicated in the pathogenesis of oxidative injury. Oxidative stress and lipid peroxidation was evaluated by ROS and TBARS level, respectively. In our experiment, PAT-induced the elevation of ROS and TBARS level in liver homogenates, indicated the involvement of oxidative stress in PAT toxicity, which is consistent with previous study in human embryonic kidney and human promyelocytic leukemia cells (Liu et al., 2007). GTP showed the ability to prevent PAT-induced TBARS, suggesting that GTP inhibited lipid peroxidation.

GSH is the major responsible regulator of intracellular redox homeostasis. Mechanistic studies on PAT-induced toxicity revealed that GSH depletion plays a critical role in PAT-induced damage (Ayed-Boussema et al., 2013; Pfeiffer et al., 2005). GSH depletion in PAT-intoxicated mice liver was noticed in our in vivo experiment, which further supported previous in vitro studies (Yang et al., 2011), however, the precise mechanism of GSH depletion was not fully understood. Although patulin–glutathione adducts were identified (Schebb et al., 2009), direct conjugation of PAT with glutathione still need to be excluded from the main excuse of GSH depletion due to the overwhelming concentration of cellular GSH compare with PAT (Pfeiffer et al., 2005). Other proposed mechanisms for PAT-induced GSH depletion include alterations in plasma membrane permeability (Riley et al., 1990) and inhibition of protein synthesis (Arafat and Musa, 1995). Since GSH depletion is associated with the pro-oxidative effects of PAT, it is reasonable to investigate whether GSH administration has protective role against PAT-induced toxicity. Indeed, previous reports have shown the protective effects of GSH, unfortunately, the elevation of GSH level cannot completely abolish the genotoxic effect of PAT (Mahfoud et al., 2002; Morgavi et al., 2003). Although the majority studies reported the depletion of GSH with PAT treatment, on the contrary, Schumacher et al. reported the increasing of GSH was observed after treatment with PAT in V79 cells (Schumacher et al., 2005). Their explanation was PAT actives antioxidative response elements and stimulates the expression of γ-glutamyl-cysteine synthetase, which enhanced biosynthesis of GSH as an adaptive response of the cells to oxidative stress ultimately.

Antioxidant defense system plays an important role in protecting liver from free radical attack. SOD and CAT are usually regarded as antioxidant enzymes that provide critical protective effects against oxidative injury. Our results demonstrated that SOD and CAT activities were both crippled with PAT-treatment, which is in accord with previous in vitro (Liu et al., 2007) and in vivo (Saxena et al., 2011) studies. Liu et al. showed the PAT-induced toxicity is highly regulated with cellular SOD and CAT expression, and overexpression of these two antioxidant enzymes led to a reduction in damage and vice versa (Liu et al., 2007). Saxena et al. showed PAT induces significant decrease in CAT, SOD and glutathione reductase (GR) activities in mice (Saxena et al., 2011). However, up-regulate activities of SOD, CAT and glutathione S-transferase (GST), but not glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx) and GR, with PAT-treatment was found in the fission yeast Schizosaccharomyces pombe (Papp et al., 2012). They claimed PAT-induced ROS accumulation triggered adaptation processes, which enhanced antioxidant enzyme activities in turn.

PAT has been reported to have genotoxic effects in vitro (Alves et al., 2000; Donmez-Altuntas et al., 2013; Glaser and Stopper, 2012; Liu et al., 2003; Mori et al., 1984; Pfeiffer et al., 1998; Zhou et al., 2009) and in vivo (de Melo et al., 2012). The mechanism studies demonstrated PAT induced DNA–DNA (Glaser and Stopper, 2012; Schumacher et al., 2006) and protein–protein (Fleiege and Metzler, 1999) cross-linking, which may associated with its mutagenic and genotoxic effects. However, there are controversial results presented regarding to genotoxic effect of PAT. PAT caused DNA single- and double strand breaks in Escherichia coli. (Lee and Roschenthaler, 1986) and in Chinese hamster ovary cell (Stetina and Votava, 1986), however, but did not cause DNA strand breaks in Chinese hamster lung cell (Schumacher et al., 2006). PAT caused the elevation in the sister chromatid exchange frequency in both Chinese hamster ovary cells (Cooray et al., 1982) and human lymphocytes (Liu et al., 2003) but not increase the SCE rate in Chinese hamster V79-E cells (Thust et al., 1982). Positive and negative results were both presented in Ames test in different strains (Alves et al., 2000; Wurglit et al., 1991). These dissimilarities may due to their different experimental models, therefore, it is worth to further investigate the genotoxic effect of PAT. Although the genotoxic effect of PAT has been illustrated in some target tissues, such as hippocampus, cortex, olfactory bulb, cerebellum, kidney, liver and bladder (de Melo et al., 2012), however, its genotoxic effect on bone marrow cells is very rare. Korte et al. demonstrated PAT showed the greater effect on inducing chromosomal damage in Chinese hamster bone-marrow cells compared with mycotoxins.
Aflatoxin B1 and Aflatoxin G1 (Korte, 1980). The bone marrow is the major hematopoietic organ in mammalian body. In the current study, PAT causes significant induction of micronucleus and chromosomal aberration formation, which is in agreement with previous study in human lymphocyte (Donmez-Altuntas et al., 2013) and mammalian cell (Alves et al., 2000). GTP, which exerted antioxidative activity in reducing hepatic ROS and TBARS level and increasing GSH content, also inhibited PAT-induced bone marrow damage, including the formation of micronucleus and chromosomal aberration. Previous study demonstrated buthionine sulfoximine (BSO), a glutathione-synthesis inhibitor which activate oxidative stress, increased PAT-induced micronucleus frequency significantly (Glaser and Stopper, 2012; Zhou et al., 2009). These results suggested PAT-induced bone marrow injury is associated with free radical attack and oxidative damage mechanism.

In conclusion, our study examined the hepatotoxicity and genotoxicity of patulin in mice and the protective role of GTP. Our results showed that PAT induces serum transaminases indicated liver injury. Also, the decrease of GSH content/antioxidant enzymes and increase of ROS/TBARS level were found in PAT-intoxicated mice liver. Consistently, we found the increase of micronucleus and chromosomal aberration in bone marrow cell. These findings suggested the pro-oxidant and genotoxic effects of PAT, and the antioxidant and anti-genotoxic effects of GTP.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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