Cytotoxic, caspase-3 induction and in vivo hepatoprotective effects of phyllanthin, a major constituent of Phyllanthus niruri

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ABSTRACT

The antiproliferative activity of phyllanthin (the major constituent in the decoction of Phyllanthus niruri L.) against human liver carcinoma HepG2 cells, the mechanism of cell death and the protective effect against carbon tetrachloride (CCl4)-induced hepatotoxicity in mice were investigated. Phyllanthin showed dose- and time-dependent growth inhibitions of HepG2 cells with the lowest EC50 value (10.16 ± 0.21 μg/ml) at 72 hours of treatment. Acute exposure to this compound exerted a significant caspase-3 expression that reached its peak after 8 hours of treatment (P < 0.001). Oral pre-treatment of phyllanthin at a dose of 120 mg/kg prior to CCl4-treatment effectively prevented the hepatic damage by reducing alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These are the indications that phyllanthin is able to act independently to inhibit HepG2 cell proliferation via apoptotic cell death and to protect from hepatic injuries. Therefore, the effects of P. niruri extracts in the treatment of liver diseases can be related to the presence of phyllanthin.

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1. Introduction

Liver diseases are commonly caused by viral infections, drug overdose, reactions to prescribed drugs and medications, prolonged or excessive exposure to environmental toxins, and long term effects of alcohol consumption (Kew, 2010; Kim, Hattori, & Phongsamran, 2010; Qua & Goh, 2011). Chronic liver diseases may also lead to the development of cancers (McClune & Tong, 2010). Although extensive studies have been conducted to understand the pathogenesis of these diseases, their effective therapies are still lacking (Song et al., 2006). Thus, these diseases should be prevented by avoiding the risk factors, maintaining a healthy balanced diet (that includes foods to strengthen the liver and improve its functions), and considering functional food supplements with hepatoprotective activity.

The decoction of Phyllanthus niruri L. (Euphorbiaceae; Synonym: Phyllanthus amarus Schumach. & Thonn.) was clinically proven as a functional food to protect the liver (Patel, Patel, * Corresponding author. School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia. Tel.: +60 4 6534095; fax: +60 4 6565125.
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It is also consumed to cleanse and detoxify the liver, and to treat liver disorders such as hepatitis and hepatocarcinoma. All these bioactivities have been proven scientifically (Patel, Tripathi, Sharma, Chauhan, & Dixit, 2011). The hepatoprotective activity was found to be associated with the major content of phyllanthin that may act synergistically with other compounds in the decoction (Krithika, Verma, Shrivastav, & Suguna, 2011). To the best of our knowledge, there were only three earlier studies that had determined the anti-hepatotoxic activity of phyllanthin. Krithika et al. (2009) and Syamasundar et al. (1985) had reported the in vitro activities on CCl4-induced toxicity in human liver carcinoma HepG2 cells, and on CCL4- and galactosamine-induced toxicity in primary cultured rat hepatocytes, respectively. In addition, a study by Krithika and Verma (2014) recently reported the in vivo activity on CCl4-induced hepatic damage in female mice (Mus musculus). However, not all compounds or extracts with anti-hepatotoxic activity may also have hepatoprotective activity. Thus far, there has only been one report on the hepatoprotective activity of phyllanthin that was determined using an in vitro system on ethanol-induced toxicity in rat hepatocytes (Chirdchupunseree & Pramyothin, 2010). Therefore, this study is the first to determine the in vivo hepatoprotective activity of phyllanthin.

Among the compounds isolated from foods that were reported to have hepatoprotective activity are lycopene from Solanum lycopersicum (tomato) fruit (Pinto, Rodríguez-Galdón, Cestro, & Macias, 2013) and hesperidin from Citrus sinensis (sweet orange) peel (Chen et al., 2013). Lycopene and hesperidin were also reported to reduce proliferation (Burgess et al., 2008), and to induce apoptosis-like cell death (Yummam et al., 2014) of HepG2 cells, respectively. To date, the antiproliferative activity of phyllanthin was only reported by Islam, Selvan, Mazumder, Gupta, and Ghosal (2008) against Ehrlich ascites carcinoma in Swiss albino mice.

The induction of apoptosis has been well recognized as a major hindrance to cancer growth and metastasis that involves the elimination of cancer cells without injuring noncancerous cells. The apoptosis mechanism is mainly regulated by the intracellular protease, especially caspase-3. Caspase-3 was identified as the main executor for morphological and biochemical alterations of apoptotic cells in both extrinsic (death receptor) and intrinsic (mitochondrial) apoptosis signaling pathways (Olsson & Zhivotovsky, 2011). The deficiency of this gene was also found to cause chemotherapeutic resistance in human cancer cells (Rebucci & Michiels, 2013). Hence, targeting only caspase-3 (the terminal protease in the apoptotic cascade) and not other upstream regulators of apoptosis could lead to a more effective therapy of cancers (MacKenzie, Schipper, & Clark, 2010). Many studies have revealed the potential of P. niruri extracts to induce caspase-3 in human breast (MCF-7), lung (A549) and liver (HepG2 and Huh-7) carcinoma cells (de Araújo Júnior et al., 2012; Lee, Jaganath, Wang, & Sekaran, 2011). However, no report on the involvement of phyllanthin in inducing caspase-3 is available.

This study was carried out (i) to assess the antiproliferative activity and induction of caspase-3 by phyllanthin on HepG2 cells, and (ii) to determine the hepatoprotective activity of the said compound against CCl4-induced hepatotoxicity in mice.

2. Materials and methods

2.1. Chemicals

Dimethylsulfoxide (DMSO), ethidium bromide, methylene blue, Tween 80 and vincristine sulfate were obtained from Sigma Aldrich, St. Louis, MO, USA. All culture media and additives (such as Earle’s balanced salt solution (EBSS), Eagle’s minimum essential medium (MEM), fetal calf serum (FCS), L-glutamine, penicillin, and streptomycin) were purchased from GE Healthcare HyClone, South Logan, UT, USA. Agarose gel, glutaraldehyde, phosphate-buffered saline (PBS) and sodium chloride (NaCl) were obtained from Amresco, Solon, OH, USA. Carbon tetrachloride (CCl4) and silymarin were purchased from Merck, Darmstadt, Germany and Simepar, Acino, Switzerland, respectively. Assay kits for serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were purchased from DiaSys Diagnostic Systems GmbH, Holzheim, Germany. Acetic acid, acetone, acetonitrile, chloroform, ethanol, ethyl acetate, hydrochloric acid (HCl), n-hexane, methanol, and sulfuric acid were purchased from Fisher Scientific, Selangor, Malaysia.

2.2. Extraction, fractionation and isolation of phyllanthin

The leaves of P. niruri (1 kg) were dried to a constant weight at 60 °C in a vacuum oven (Memmert, Frankfurt, Germany). The dried sample was ground for 1 min using a grinder to yield a fine powder (80 mesh of particle size). The sample was extracted using chloroform in a Soxhlet apparatus. The dried extract was separated by silica gel column chromatography (60–120 mesh) using a gradient of n-hexane and ethyl acetate (from ratio 9:1 (v/v) to 1:9 (v/v)) as described by Tripathi, Verma, Gupta, Gupta, and Khannuja (2006). Phyllanthin was purified by crystallization (3 times) in petroleum ether (Fig. 1). The identification of phyllanthin was further confirmed by ultra violet (UV), infra red (IR) and ultra performance liquid chromatography (UPLC) analyses in comparison with the standard of phyllanthin (ChromaDex, Irvine, CA, USA). The UV and IR spectra were detected by a CARY 50 Conc-UV visible spectrometer (Santa Clara, CA, USA) and a Perkin Elmer system 2000 FT-IR spectrometer with KBr discs (Waltham, MA, USA), respectively. The UPLC analysis was carried out using the method described by Murugaiyah and Chan (2007) with some modifications using an Acquity UPLC system (Waters, Milford, MA, USA). The compound was dissolved in 99.9% (v/v) DMSO to prepare a stock solution at a concentration of 10 mg/ml. The
stock solution was serially diluted to eight different concentrations (10–0.078 mg/ml) for the antiproliferative test.

2.3. Cell culture

The HepG2 (ATCC, HB-8065) cells were obtained from American Type Cell Culture (ATCC) and grown in MEM/EBSS medium.

2.4. Antiproliferative analysis

The methylene blue assay was carried out as previously described (Ooi et al., 2014). Briefly, 100 μl of cells were plated at a density of approximately 6000 cells/well into a 96-well plate. The cells were treated with different concentrations of phyllanthin by adding 1 μl of the serially diluted compound into each well. Positive control cells were treated with vincristine sulfate and negative control cells were cultured in 0.5% (v/v) FCS-containing medium alone. The final concentration of DMSO in all wells was kept less than 1% (v/v). After 24, 48 and 72 hours of incubation with the compound, the surviving cells were fixed with 2.5% (v/v) glutaraldehyde for 15 min. One hundred microliters of 0.05% (w/v) methylene blue solution was added to stain the fixed cells for 15 min. The absorbance was read at 650 nm using a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The percentage of growth inhibition was calculated according to the following equation:

\[
\text{Growth inhibition (\%)} = \left( \frac{(\text{absorbance of negative control} - \text{absorbance of sample})}{\text{absorbance of negative control}} \right) \times 100\%.
\]

The EC_{50} values for growth inhibition were derived from a nonlinear model (curvefit) based on a sigmoidal dose–response curve (variable) and computed using GraphPadPrism, San Diego, CA, USA. All experiments were performed in six replicates.

2.5. Determination of caspase-3 gene expression

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed as previously described by Ooi et al. (2014) to determine the caspase-3 expression level. The cells were then treated at different incubation periods with phyllanthin using the concentration of EC_{50} at 72 hours (10.16 ± 0.21 μg/ml). After the treatment, total cellular RNA was isolated using Tri-Reagent LS according to the manufacturer’s instructions (Molecular Research Center, Cincinnati, OH, USA). The amplification products were separated on 1.2% (w/v) agarose gel and stained with ethidium bromide. Gene expressions signaling at each time interval were quantified using GeneTools analysis software on a GENEGENIUS gel documentation system (Syngene, Cambridge, UK). The signals of caspase-3 were normalized against β-actin and the ratio in untreated cells (negative control) was assigned as 1.

2.6. Experimental animals

Male Mus domesticus (21–32 g) were obtained from the Animal House Facility, Universiti Sains Malaysia and used throughout the study. They were housed in a 12 hours light–dark cycle at 25 ± 2 °C with a relative humidity of 30–60%, and were fed standard mice pellets and water ad libitum. The animals were allowed to acclimatize to laboratory conditions for 7 days before the commencement of experiments. The experiments were conducted in accordance with the Animal Ethics Committee Guidelines of Universiti Sains Malaysia (Protocol acceptance number: USM/PPSF/50(091)JLD2).

2.7. Hepatoprotective analysis

For this analysis, the compound was reconstituted with Tween-80. In order to ensure that there are no toxic effects of the compound, the doses used in this study were selected according to our preliminary toxicity evaluation. The mice were randomly divided into six groups consisting of six animals in each group. Group I received Tween-80 once a day orally for 6 days and served as the normal control. Group II received CCl4 at a dose of 12.8 μl/kg body weight (b.wt.) by gastric gavages only on days 5 and 6 to induce hepatotoxicity. Mice in Groups III–V were pre-treated with phyllanthin for 4 days, at doses of 60, 120, 180 mg/kg b.wt., respectively. On days 5 and 6, the mice were still treated with phyllanthin and after an hour of treatment, they were administered with CCl4 (12.8 μl/kg b.wt.) Group VI (positive control) received silymarin (35 mg/kg b.wt.) for 6 days and were administered with the CCl4 on days 5 and 6. The mice were anesthetized with diethyl ether, 24 hours after the last oral dose of each experiment. Blood and liver samples were collected for biochemical and histopathological examinations. The serum was separated by centrifugation at 1000 rpm for 5 min and used for the measurement of biochemical markers namely, ALT and AST activities using commercially available assay kits (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). The absorbance of reaction was determined at 340 nm. For the histopathological studies, the livers were quickly removed after autopsy, blotted with filter paper, weighed and fixed in 10% neutral buffered formalin. The paraffin sections were prepared and stained with hematoxylin and eosin (H&E) dye and examined using light microscopy (Olympus BH2, Shinjuku-ku, Tokyo, Japan).

2.8. Statistical analysis

The results were expressed as mean ± standard deviation (SD) using GraphPadPrism. Analysis of variance was determined by one-way ANOVA (GraphPadPrism). Significant differences between the means were calculated according to the Duncan’s multiple range test. Differences at P < 0.05 and P < 0.001 were considered statistically significant.

3. Results

3.1. Antiproliferative effects of phyllanthin against HepG2 cells

In the present study, the antiproliferative effects of the phyllanthin against HepG2 cells were evaluated at incubation periods of 24, 48 and 72 hours. As indicated in Fig. 2, more
than 55% of the growth inhibition of HepG2 cells was observed at higher concentrations (50–100 μg/ml) for all the incubation periods. A time dependent inhibition of HepG2 cells was also observed. The range of concentrations with significant growth inhibition was increased from 6.25–100 μg/ml at 24 hours to 3.125–100 μg/ml at 48 and 72 hours (P < 0.05). Thus, the EC50 values were decreased as the incubation periods were increased from 24 (39.53 ± 6.47 μg/ml) to 48 (18.04 ± 2.44 μg/ml) and 72 (10.16 ± 0.21 μg/ml) hours (Table 1). The lowest EC50 value was obtained after 72 hours of treatment. Thus, this concentration was used to determine the mechanism of cell death.

### 3.2. Induction of caspase-3 gene expression in HepG2 cells by phyllanthin

The semi-quantitative RT-PCR showed that the mRNA expression of caspase-3 in HepG2 cells was significantly induced as early as 4 hours of treatment (nearly 6.0-fold increase) (P < 0.001) (Fig. 3) with phyllanthin using the concentration of EC50 at 72 hours (10.16 ± 0.21 μg/ml). The gene expression was then elevated continuously and reached its peak after 8 hours of treatment (approximately 7.0-fold increase). This was followed by a gradual decrease at 12 hours (4.0-fold decrease) and further reduction in the next 4 hours of post stimulation. It declined drastically to the level below the baseline at 24 hours.

### Table 1 – EC50 values of phyllanthin against HepG2 cells at different incubation periods.

<table>
<thead>
<tr>
<th>Incubation periods</th>
<th>EC50 (μg/ml)</th>
</tr>
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<tbody>
<tr>
<td>24 hours</td>
<td>39.53 ± 6.47</td>
</tr>
<tr>
<td>48 hours</td>
<td>18.04 ± 2.44</td>
</tr>
<tr>
<td>72 hours</td>
<td>10.16 ± 0.21</td>
</tr>
<tr>
<td>Vincristine sulfate</td>
<td>0.14 ± 0.006</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of six replicates (n = 6).
When compared with control cells, the caspase-3 expression was proven to be significantly different from 4 to 24 hours post-stimulation (P < 0.05). Therefore, the results clearly proposed that the mechanism of cell death elicited by the phyllanthin was mediated via the activation of caspase-3.

3.3. Hepatoprotective effects of phyllanthin on CCl₄ intoxicated mice

As depicted in Table 2, the serum levels of ALT and AST, which are used commonly as biochemical markers for the evaluation of early hepatic injury, were elevated at 210.35 and 332.59 U/l, respectively in the CCl₄-treated mice. Meanwhile, the administration of phyllanthin at doses of 120 and 180 mg/kg effectively attenuated (at least 2-fold reduction) the CCl₄-induced elevation of serums ALT and AST (P < 0.05). The decreasing levels of serums were found to be ranging between 74–83 and 62–63%, respectively. These hepatoprotective effects were comparable to the standard drug, silymarin, which shows nearly 66% decrease of the serum levels. This clearly revealed the effectiveness of phyllanthin to limit the leakage of the ALT and AST enzymes.

Histopathological examinations further confirmed the protective properties of phyllanthin and provided the visual evidence for its hepatoprotective efficacy. Histology of the liver sections from the normal group showed typical hepatic architecture with well-preserved cytoplasm, prominent nucleus and radiately arranged hepatocytes around the central vein (Fig. 4A). In contrast, the CCl₄ treated liver sections showed an excessive parenchymal disarrangement, such as ballooning degeneration, infiltration of inflammatory cells and loss of cellular boundaries (Fig. 4B). The hepatic lesions were markedly prevented by pre-treatment with phyllanthin at 120 mg/kg (Fig. 4D). It displayed a more or less normal lobular pattern with a mild degree of necrotic zones, lymphocyte infiltration, hepatocellular degeneration and is almost comparable to the normal control and silymarin treated (Fig. 4C) groups. This clearly indicated that the compound affords a comparable protective effect with its standard reference, silymarin in the CCl₄-mediated hepatic injury.

<p>| Table 2 – Effects of the phyllanthin and silymarin on alanine transaminase (ALT) and aspartate transaminase (AST) levels in CCl₄-intoxicated mice. |
|-----------------------------------|-------------|-------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (1 U/l)</th>
<th>AST (1 U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I, normal</td>
<td>16.16 ± 2.59</td>
<td>54.65 ± 1.42</td>
</tr>
<tr>
<td>Group II, CCl₄ treated</td>
<td>244.24 ± 59.01*</td>
<td>565.21 ± 136.51*</td>
</tr>
<tr>
<td>Group III, pre-treatment</td>
<td>199.69 ± 99.70*</td>
<td>612.85 ± 108.41*</td>
</tr>
<tr>
<td>phyllanthin 60 mg/kg + CCl₄</td>
<td>62.26 ± 5.52b</td>
<td>92.45 ± 7.85b</td>
</tr>
<tr>
<td>Group IV, pre-treatment</td>
<td>199.69 ± 99.70*</td>
<td>612.85 ± 108.41*</td>
</tr>
<tr>
<td>phyllanthin 120 mg/kg + CCl₄</td>
<td>62.26 ± 5.52b</td>
<td>92.45 ± 7.85b</td>
</tr>
<tr>
<td>Group V, pre-treatment</td>
<td>90.24 ± 32.16b</td>
<td>210.31 ± 40.50b</td>
</tr>
<tr>
<td>phyllanthin 180 mg/kg + CCl₄</td>
<td>81.49 ± 15.44b</td>
<td>188.98 ± 21.96b</td>
</tr>
<tr>
<td>Group VI, silymarin</td>
<td>199.69 ± 99.70*</td>
<td>612.85 ± 108.41*</td>
</tr>
<tr>
<td>35 mg/kg + CCl₄</td>
<td>62.26 ± 5.52b</td>
<td>92.45 ± 7.85b</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of six replicates (n = 6).
* P < 0.05 vs normal control.
b P < 0.05 vs CCl₄ control.

4. Discussion

Even though phyllanthin is considered to be the main contributor to the hepatoprotective property of P. niruri, its abilities to trigger the apoptosis-based growth inhibition on HepG2 carcinoma and in vivo hepatoprotective activity of CCl₄-intoxicated mice were unveiled for the first time in this study. Both of these bioactivities are important mechanisms involved in the treatment and prevention of liver disorders. The isolated phyllanthin was subjected to the methylene blue assay for the antiapopterferative evaluation, and RT-PCR analysis to determine the mRNA expression level of the apoptotic gene. The hepatic enzymes were measured and histopathological analyses were performed to investigate the hepatoprotective activity.

Phyllanthin was found to possess a concentration- and time-dependent inhibition of HepG2 cells in the preliminary antiproliferative evaluation. This compound displayed a marginal inhibitory reaction at the early stages of treatment (24 hours) and the activity gradually increased when the incubation time was increased to 48 and 72 hours, which reduced nearly 2.0-fold and 4.0-fold, respectively of EC₅₀ values than that of 24 hours (Table 1). This clearly indicated that a lower dose of the compound is adequate to abrogate 50% of the HepG2 cell growth when a longer period of exposure is applied. This might be due to the cumulative cytotoxic response of the compound toward HepG2 cells. The antiproliferative potential of this compound is mainly due to the occurrence of the methoxy moieties in the chemical structure, which has been associated with the anticancer activities against various human carcinoma cells (Orfila et al., 2000). Somanabandhu et al. (1993) speculated that phyllanthin acts as a cytotoxic enhancer in vinblastine-mediated multidrug resistant KB oral carcinoma cells through the displacement of vinblastine bound to the cell membrane vesicles, probably by the interaction with P-glycoprotein.

The concentrations used in this study are in fact higher than that of Krithika et al. (2009) against the same cell lines. This means that phyllanthin at lower concentrations can act as an anti-hepatotoxin and at higher concentrations can act as a cytotoxic compound. Other compounds from foods were also found to have these properties in the HepG2 cells. For example, lycopene, at lower concentrations was reported to possess antigenotoxicity and antimutagenicity (Scolastici et al., 2008), and to attenuate arachidonic acid toxicity (Xu, Leo, & Lieber, 2003a) and alcoholic apoptosis (Xu, Leo, & Lieber, 2003b), whereas at higher concentrations, it was found to reduce the cell proliferation (Burgess et al., 2008).

Different mRNA expressions of caspase-3 were observed in HepG2 cells treated with phyllanthin for 24 hours. The peak expression of this gene was significantly observed at 8 hours (P < 0.05), indicating that caspase-3 was highly induced at the early phase of the treatment with this compound. This might lead to the amplifying proteolytic cascade of caspases and triggering of the apoptotic morphological alterations and the DNA fragmentation (Salvesen & Riedl, 2008). The sustainability of this gene expression until the late treatment periods could be due to its involvement in the late phase of apoptotic pathway as a downstream stimulator, which is activated after a series of upstream cellular processes during apoptosis (Chowdhury, Tharakan, & Bhat, 2008). In agreement with our findings, the
marked caspase-3 activities in human liver carcinoma cells (HepG2 and Huh-7) were also induced by the spray-dried extract of *P. niruri* (de Araújo Júnior et al., 2012). It is worth noting that the apoptosis triggering potential of phyllanthin might be ascribed to its main dibenzylbutane skeleton, which has been pointed out to evade caspase-3 activity and DNA fragmentation in Huh-7 cells (Mansoor, Ramalho, Rodrigues, & Ferreira, 2012). These findings provide strong evidence to support the role of this compound as the main apoptosis inducer of *P. niruri* extracts in inhibiting the growth of human liver carcinoma cells.

The results obtained from our *in vivo* hepatoprotective study also help to further verify the earlier *in vitro* study of the hepatoprotective potential of phyllanthin (Chirdchupunseree & Framyothin, 2010). CCL4 has been extensively highlighted as a hepatotoxicant and its metabolites such as trichloromethyl radicals are reported to be involved in the pathogenesis of liver damage by disrupting the structure and function of lipid and protein macromolecule in the cell membrane (Clawson, 1989). This might lead to hepatic lipid peroxidation and eventually result in the release of ALT and AST enzymes. The abnormally high levels of these intracellular enzymes that were observed in the present study (*Table 2*) were the consequences of CCL4-induced liver dysfunction, indicating that these enzymes leaked out from the liver into the circulatory system at the instance of tissue damage.

Meanwhile, treatment with phyllanthin especially at a dose of 120 mg/kg exhibited a significant restoration of the altered biochemical parameters in the CCL4-intoxicated mice (*P* < 0.05). Lower ALT and AST levels than that of silymarin, a standard hepatoprotective reference, were measured. It is strongly proposed that the compound affords better protection against CCL4. This hepatic protective effect was also reflected by the histopathological examination. The severe hepatic impairments induced by CCL4 were effectively attenuated to normal by pre-treatment with this compound, which was comparable to that of the silymarin-treated group.

This implies that the compound could increase the stabilization of plasma membrane and effectively alleviate biliary dysfunction thereby preserving the structural integrity of cells as well as the repair of CCL4-induced liver damage. The results from this study also provide further corroboration of the previous studies that have reported the *in vivo* anti-hepatotoxic activity of phyllanthin and phyllanthin-standardized ethanolic extracts of *P. niruri* (Krithika & Verma, 2014; Krithika et al., 2011). According to Krithika et al. (2009), the antioxidative property of this compound was important in scavenging CCL4-induced free radicals and protecting the hepatic cell membrane from destruction. It was reported that the methoxy groups of the compound might enhance the hydrogen donating capacities and radical scavenging activities (de Pinedo, Peñalver, & Morales, 2015).
suggesting that these moieties indirectly assist in neutralizing and ameliorating the radical injury of intoxicated hepatic cells.

5. Conclusions

Our study revealed the therapeutic potential of phyllanthin for treating hepatic-related diseases by inhibiting HepG2 cell proliferation, inducing apoptosis in HepG2 via caspase-3-dependent cell death mechanism and protecting against CCl4-induced hepatotoxicity. The marked hepatoprotective activity of this compound is also suggested to be associated with the activation of caspase-3 gene, which plays a vital role in modulating and preventing excessive or deregulated apoptosis-caused chronic liver injuries.

Conflict of interest

The authors declare that there are no conflicts of interest.

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