

## Research Article

# In Vitro Investigation of the Anticancer Activity of *Peucedanum praeruptorum* Dunn Extract on HepG2 Human Hepatoma Cells

Shiwen Hu,<sup>1,2</sup> Pan Wang,<sup>3</sup> Yongchun Chen,<sup>4</sup> Gang Kuang,<sup>2</sup> Cun Wang,<sup>2</sup> Jing Luo,<sup>2</sup> and Shaocheng Chen<sup>1,2</sup> 

<sup>1</sup>Chongqing Field Scientific Observation and Research Station for Authentic Traditional Chinese Medicine in the Three Gorges Reservoir Area, Chongqing University of Education, Chongqing 400067, China

<sup>2</sup>College of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, China

<sup>3</sup>Department of Traumatology, Chongqing University Central Hospital, Chongqing Emergency Medical Center, Chongqing 400013, China

<sup>4</sup>Wuxi County Agricultural Products Quality and Safety Supervision Station, Chongqing 405899, China

Correspondence should be addressed to Shaocheng Chen; [cque@foxmail.com](mailto:cque@foxmail.com)

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The anticancer activity of *Peucedanum praeruptorum* Dunn extract (PPDE) was investigated *in vitro* in the HepG2 human hepatoma cell line and compared to normal human liver cells (L02 cells). The effect of the PPDE on the proliferation of the cells was measured by MTT assays, and the levels of enzymes and small molecules implicated in oxidative stress regulation were measured using specific reagent kits. The expression levels of genes implicated in apoptosis (Bax, Bcl-2, caspase-3, caspase-8, and caspase-9) and oxidative stress (SOD1 and SOD2) were quantified by RT-qPCR. Lastly, HPLC was employed to analyze the composition of the PPDE. PPDE was found to significantly inhibit the proliferation of HepG2 cells but had little effect on the proliferation of normal liver cells. PPDE increased the levels of reactive oxygen species and malonaldehyde, a lipid peroxidation product, in HepG2 cells, and it reduced the activities of antioxidant enzymes, as well as the levels of  $\gamma$ -GCS and reduced glutathione (GSH), suggesting that it inhibited the ability of cancer cells to regulate intracellular oxidative stress. PPDE also increased the expression of the genes encoding Bax, caspase-3, caspase-8, and caspase-9 and decreased the expression of Bcl-2, SOD1, and SOD2 in HepG2 cells, suggesting that PPDE induced the apoptosis of the liver cancer cells. HPLC analysis identified that the components of PPDE included caffeic acid, isochlorogenic acid C, myricetin, baicalin, luteolin, and kaempferol, all of which have demonstrated antioxidant properties.

## 1. Introduction

*Peucedanum praeruptorum* Dunn is a member of the *Apiaceae* family of aromatic flowering plants and is native to China [1]. It is a perennial herb whose roots and stems are prepared into traditional Chinese medicines (TCMs) [2] because it can relieve the symptoms of fever, cold, cough, and bronchitis and act as an expectorant, mainly due to the presence of coumarin compounds [3–5]. In particular, angular dihydropyrano coumarins, such as praeruptorin A and ulopterol, that are extracted from *P. praeruptorum* Dunn have been used as raw materials for various health products

[6]. Similarly, ulopterol extracted from *P. praeruptorum* Dunn is formulated into health drinks [7]. *P. praeruptorum* Dunn can also be mixed with plants such as *Platycodon grandiflorus* and green tea to prepare herbal teas for therapeutic applications. Furthermore, *P. praeruptorum* Dunn plants are commonly cooked and mixed with honey for direct consumption [6, 7].

Previous studies have demonstrated the anticancer effects of *P. praeruptorum* Dunn extracts (PPDE) in *in vitro* rectal and cervical cancer models [8]. These anticancer properties might involve the ability of the chemical components of the extract to degrade reactive oxygen species

(ROS), including superoxide anions ( $O_2^-$ ), peroxide ions ( $O_2^{2-}$ ), hydroxyl radicals ( $^{\bullet}OH$ ), hydrogen peroxide ( $H_2O_2$ ), peroxyxynitrite ( $ONOO^-$ ), organic peroxy free radicals ( $ROO^{\bullet}$ ), lipid peroxy free radicals ( $LOO^{\bullet}$ ), and other by-products of cellular respiration. It is commonly accepted that excessive ROS buildup in cells induces oxidative stress, which can harm or even kill cells. However, despite being naturally resistant to oxidative stress, cancer cells might succumb to ROS-induced death if this resistance is lost [9]. Excessive ROS production can damage cancer cells by increasing the peroxidation of polyunsaturated fatty acids in cell membranes and degrading other biological macromolecules such as proteins and nucleic acids [10]. Antioxidant compounds may therefore aid in the prevention of cancer by reducing the capacity of cells to defend themselves against oxidative stress.

Inducing apoptosis has been shown to be an effective method for preventing cancer cells from proliferating. Apoptosis can be triggered by external agents that regulate the expression of specific genes, including those that encode members of the Bcl-2 family of cell death-regulating proteins. In addition, activating specific caspases or increasing the expression of the genes that encode these caspases can also result in apoptosis in cancer cells [11]. Therefore, the bioactive ingredients found in health products or edible plants may have the ability to kill cancer cells by modulating the expression of genes from the Bcl-2 and caspase families, which are essential for apoptosis induction [12, 13].

The objective of this research was to explore the possible anticancer effect of PPDE on liver cancer through *in vitro* experiments. By doing so, we aim to establish a theoretical foundation for the utilization and advancement of PPDE. More specifically, our study will concentrate on assessing the application of PPDE in *in vitro* cancer models, as liver cancer is a prevalent malignancy in Asia and poses a substantial threat to individuals' well-being [14].

## 2. Materials and Methods

### 2.1. PPDE Extraction

*P. praeruptorum* Dunn (Wenyaotang Trading Co., Ltd., Yulin, Guangxi, China) was freeze-dried and ground into a fine powder. A 100 g sample of the powder was heated in 1000 mL of an 80% (v/v) aqueous ethanol solution at 50°C for 1 hour. The suspension was filtered, and the extraction process was repeated on the remaining solid residue. The soluble filtrates were collected and combined, and the ethanol was removed by rotary evaporation to obtain the PPDE. The ground powder was extracted three times in multiple repetitions to obtain variations in the composition of the extract.

**2.2. Determination of Cell Viability.** L02 and HepG2 cells were maintained in Dulbecco's modified Eagle medium (DMEM, Invitrogen, New York, USA) containing 10% inactivated calf serum in a humidified incubator at 37°C with a 5%  $CO_2$  atmosphere. After one week of culture, the medium was

discarded, and the cells were lifted from the plates with 0.05% trypsin-EDTA (Invitrogen). The cells were collected by centrifugation at 3000 rpm and then resuspended in each well at a density of  $1 \times 10^4$  cells/mL. This suspension was inoculated into a 96-well culture plate at a volume of 200  $\mu$ L per well. After the cells were adherent, the upper liquid medium was discarded. Then, aliquots (200  $\mu$ L) of PPDE of different concentrations (50, 100, and 200  $\mu$ g/mL) were added to each well, and the cells were incubated for an additional 48 h. Meanwhile, doxorubicin hydrochloride (1  $\mu$ M) was added to each well, and the cells were incubated for another 48 h. After discarding the upper liquid medium, 200  $\mu$ L of 5 mg/mL MTT (Invitrogen) was added to each well, and the cells were incubated for 4 h. Finally, 200  $\mu$ L of DMSO was added to each well to dissolve the purple-colored formazan product, and the plates were allowed to shake for 30 min. The absorbance of the solution in each well was measured at 570 nm, and rates of cell survival were calculated. The control group and PPDE treatments were repeated in 8 parallel experiments. The survival rate was calculated as a percentage with the formula  $OD_p/OD_c \times 100$ , wherein  $OD_p$  is the absorbance of the PPDE-treated well, and  $OD_c$  is the absorbance of the control well. The proliferation inhibition rate was calculated as a percentage using the formula  $(OD_c - OD_p)/OD_c \times 100$  [12].

**2.3. Determination of Intracellular Malondialdehyde (MDA) Production.** After the cells were treated as described in Section 2.2, a thiobarbituric acid colorimetric assay was employed to measure MDA production in the cells [15]. After rinsing with phosphate buffered saline (PBS), all cells were collected with a cell scraper and added to a precooled cell lysis solution. Then, a sample (500  $\mu$ L) of the cell lysis supernatant was mixed with 15% trichloroacetic acid and 0.67% thiobarbituric acid (400  $\mu$ L) and added to a 5 mL glass test tube. The mixture was thoroughly mixed and incubated for 20 min in a 95°C water bath. After cooling, 3 mL of isopropyl alcohol was added to extract the colored compound, and the absorbance of the solution was measured at 532 nm. The total protein content of the cells was quantified using a commercially available kit (NanJing JianCheng Bioengineering Institute, Nanjing, Jiangsu, China).

**2.4. Determination of Intracellular ROS Content.** After the cells were treated in a 6-well cell culture plate as described in Section 2.2, DMEM containing 20  $\mu$ mol/L 2',7'-dichlorodihydrofluorescein diacetate was added, and the mixture was incubated for 20 min at 37°C. The treated cells were washed twice with cold PBS [15] and resuspended in  $x$ , and a FLUOstar OPTIMA fluorimeter was used to measure the fluorescence intensity ( $\lambda_{ex}$ : 485 nm;  $\lambda_{em}$ : 530 nm) to determine the relative ROS content.

**2.5. Determination of Intracellular Antioxidant Enzyme Activity, GSH Content, and  $\gamma$ -GCS Activity.** Cells were seeded into 6-well plates at a density of  $2 \times 10^5$  cells/well and then treated as described in Section 2.2 [16]. After the treatment, the cell lysates were assayed for superoxide dismutase

(SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), and gamma-glutamylcysteine synthetase ( $\gamma$ -GCS) activity using commercial kits (NanJing JianCheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the instructions in each kit. Enzyme activities were expressed as enzyme-specific activity units (U/mg protein), while the GSH content and  $\gamma$ -GCS activity were expressed as units of  $\mu\text{mol/mg}$  protein, and these values were corrected for total cellular protein content.

**2.6. Quantitative RT-PCR.** Total RNA was extracted from the cells using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and the concentration of total RNA in each sample group was adjusted to the same level with  $x$  after determination of purity by UV spectrophotometry. Equivalent amounts of mRNA ( $2\ \mu\text{g}$ ) from each treatment group were added to sterilized PCR tubes containing oligo-dT18, RNase, dNTP, and MLV enzyme ( $1\ \mu\text{L}$  each) as well as 5x reaction buffer ( $10\ \mu\text{L}$ ). The cDNA was synthesized in three steps:  $37^\circ\text{C}$  for 120 min,  $99^\circ\text{C}$  for 4 min, and  $4^\circ\text{C}$  for 3 min [15]. Then, the mRNA expression was quantified by real-time fluorescent quantitative PCR (RT-qPCR). A sample of the cDNA ( $2\ \mu\text{L}$ ), upstream and downstream primers ( $10\ \mu\text{mol/L}$ , Thermo Fisher Scientific, Waltham, MA, USA), SYBR Premix Ex Taq II ( $10\ \mu\text{L}$ ), ROX Reference Dye ( $0.4\ \mu\text{L}$ ), and sterilized double-distilled water ( $5.6\ \mu\text{L}$ ) were added to the mix solution to yield a total reaction volume of  $20\ \mu\text{L}$ . The reactions were performed in a StepOne Plus PCR apparatus (Thermo Fisher Scientific). Reaction conditions included 40 cycles at  $95^\circ\text{C}$  for 35 s,  $55$ – $59^\circ\text{C}$  for 30 s,  $95^\circ\text{C}$  for 15 s, and  $60^\circ\text{C}$  for 60 s, after which a final extension at  $95^\circ\text{C}$  for 15 s was performed. The cDNA samples of each gene were amplified 3 times in parallel, and the expression level of the target gene was reported as the mean of the three  $C_t$  values. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference [17, 18].

**2.7. Chromatographic Analysis Method (HPLC).** High-performance liquid chromatography (HPLC, UltiMate 3000, Thermo Fisher Scientific) was utilized to analyze the composition of sample compounds. Standard samples ( $2\ \text{mg}$ , Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) were placed in separate centrifuge tubes each containing  $5\ \text{mL}$  of  $x$ . Then,  $2\ \text{mL}$  of methanol was added to each tube, and the mixture was thoroughly mixed to prepare standard stock solutions. Test solutions were prepared by dissolving standard substances and extracts from the samples in methanol. The compositions of the PPDE samples were determined using the following chromatographic parameters: diode array detector;  $4.6\ \text{mm} \times 150\ \text{mm}$ ,  $5\ \mu\text{m}$  Acclaim<sup>TM</sup>120 C18 column; mobile phase consisting of methanol and 0.5% acetic acid; detection wavelength set at  $359\ \text{nm}$ ; column temperature maintained at  $35^\circ\text{C}$ ; flow rate set at  $0.6\ \text{mL/min}$ ; and sample injection volume of  $20\ \mu\text{L}$ . A gradient elution using acetonitrile (mobile phase C) and 0.5% acetic acid (mobile phase B) was used, the conditions of which are shown in Table 1.

TABLE 1: Flow phase gradient elution conditions.

$t$ (min)	$V$ (mobile phase B) (%)	$V$ (mobile phase C) (%)
0	88	12
30	60	40
35	0	100
40	0	100

**2.8. Statistical Analysis.** The SAS9.1 statistical software was used to analyze the mean value of the results of three parallel experiments. The Tukey post hoc test using one-way analysis of variance (ANOVA) was used to analyze the statistical differences between groups. A  $P < 0.05$  was considered statistically significant.

### 3. Results

**3.1. Compound Composition of PPDE.** Analysis of the composition of the PPDE by HPLC found that the PPDE comprised six different components: caffeic acid, isochlorogenic acid, myricetin, baicalin, luteolin, and kaempferol (Figure 1).

**3.2. Effects of PPDE on the Proliferation of L02 Normal Liver Cells and HepG2 Hepatoma Cells.** As shown in Figure 2, incubation of L02 cells with different concentrations of PPDE ( $0$ – $200\ \mu\text{g/mL}$ ) did not have any detectable effect on the proliferation of normal human liver cells, with the growth rate of L02 cells remaining consistently close to 100%. In contrast, the proliferation of HepG2 hepatocellular carcinoma cells was negatively correlated with the concentration of PPDE. Higher concentrations of PPDE treatment resulted in lower rates of proliferation for HepG2 cells. Comparatively, doxorubicin, a chemotherapeutic drug, demonstrated significantly higher potency against HepG2 cells compared to PPDE; this strong inhibition of cell proliferation was also observed in the L02 cells. These findings suggested that PPDE inhibited the proliferation of cancer cells without having an apparent impact on normal cells at the concentrations tested. In contrast, while doxorubicin potently inhibited the proliferation of liver cancer cells, it also displayed high toxicity. These results highlighted the advantages of PPDE, as it exhibited minimal toxicity toward normal cells and demonstrated *in vitro* anticancer effects in live cells. Therefore,  $50$ ,  $100$ , and  $200\ \mu\text{g/mL}$  PPDE were selected for further study. Using MTT assays, treatment of HepG2 hepatocellular carcinoma cells with PPDE at  $50$ ,  $100$ , and  $200\ \mu\text{g/mL}$  resulted in growth inhibition of  $15.5 \pm 1.6\%$ ,  $50.4 \pm 2.5\%$ , and  $78.4 \pm 2.4\%$ , respectively, relative to untreated cells. PPDE, therefore, was found to inhibit the growth of HepG2 hepatocellular carcinoma cells *in vitro*.

**3.3. Effects of PPDE on MDA Content and ROS Level in HepG2 Hepatoma Cells.** As shown in Figure 3, the MDA content in HepG2 HCC cells after PPDE treatment was significantly higher compared to that in normal HepG2 HCC cells ( $P < 0.05$ ), with the highest MDA content

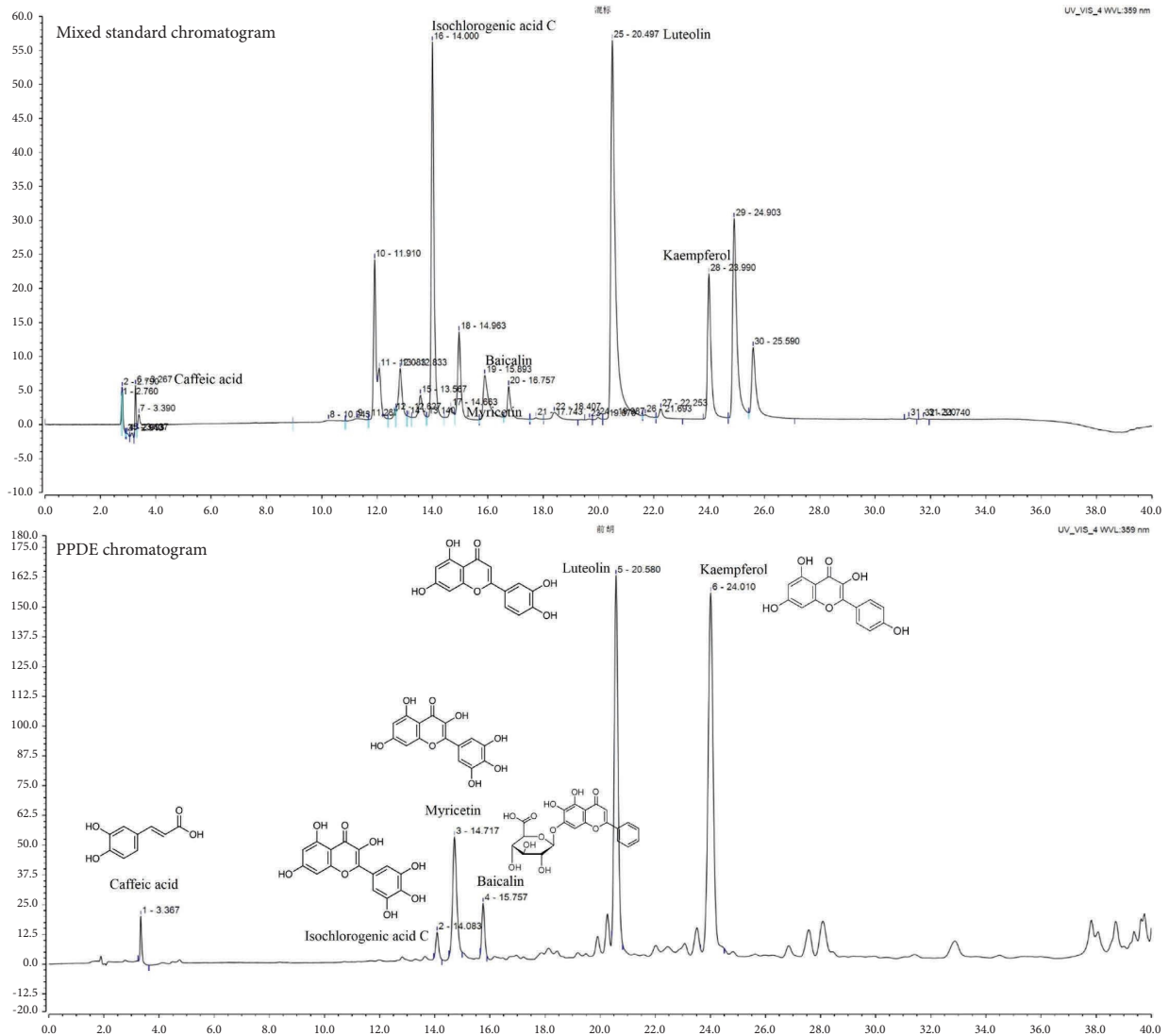


FIGURE 1: Compound composition of the *Peucedanum praeruptorum* Dunn extract (PPDE) detected by HPLC.

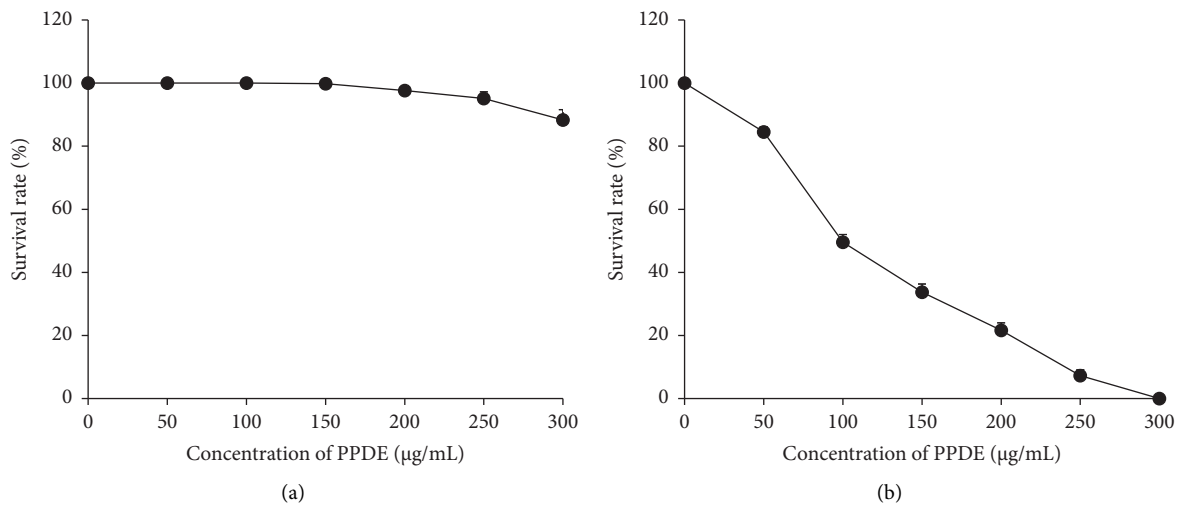


FIGURE 2: Continued.

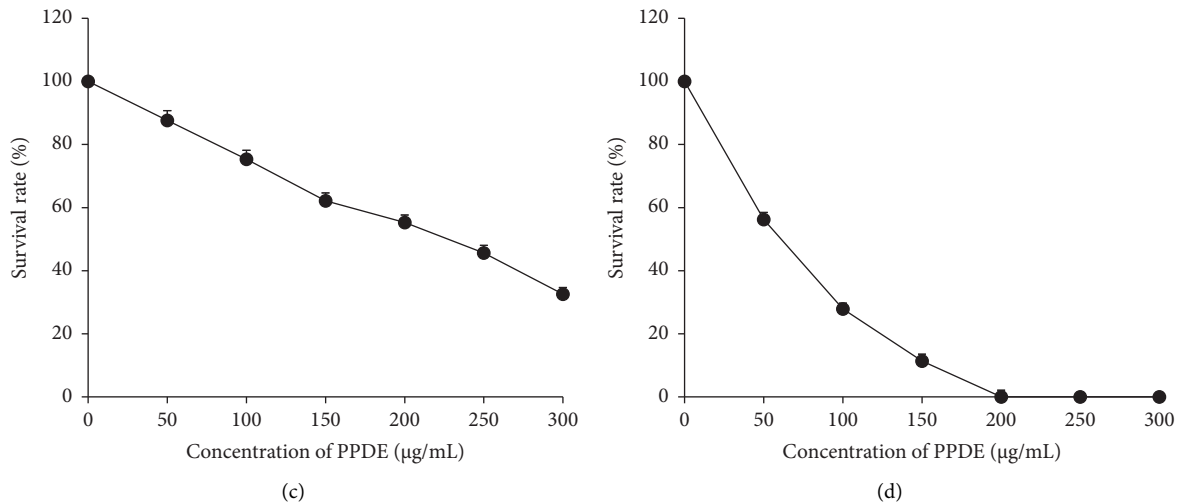


FIGURE 2: Effects of *Peucedanum praeruptorum* Dunn extract (PPDE) and positive control drug of doxorubicin on proliferation of the human normal liver cell line L02 and HepG2 human hepatoma cells ( $n=8$ ). (a) PPDE-treated L02 cells, (b) PPDE-treated HepG2 cells, (c) doxorubicin-treated L02 cells, and (d) doxorubicin-treated HepG2 cells.

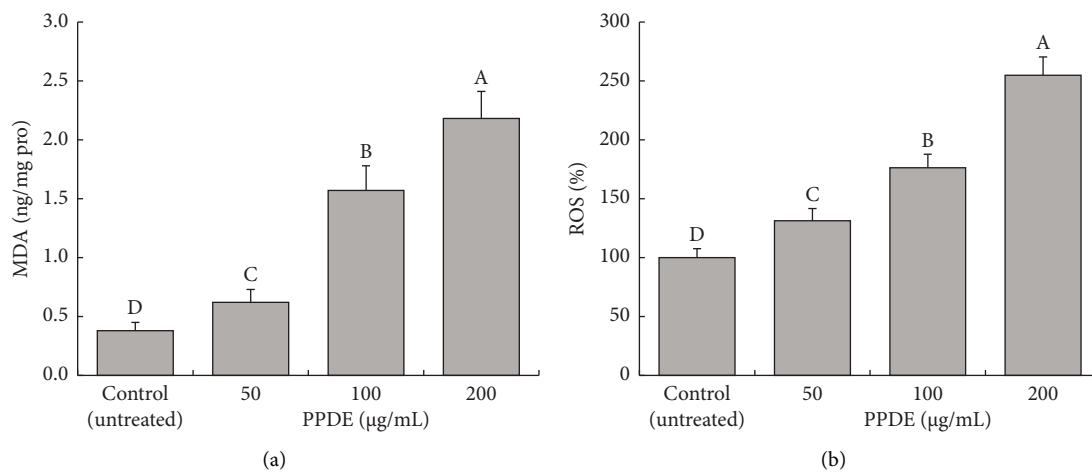


FIGURE 3: Effects of *Peucedanum praeruptorum* Dunn extract (PPDE) on the levels of MDA (a) and ROS (b) in HepG2 human hepatoma cells ( $n=8$ ). <sup>A–D</sup>Different lowercase letters indicate significant differences between the mean values of each group ( $P < 0.05$ ).

observed in the cells treated with 200 µg/mL PPDE. Similarly, after treatment with different concentrations of PPDE for 24 h, the levels of ROS in HepG2 HCC cells increased as the PPDE concentration increased in a dose-dependent manner.

**3.4. Effects of PPDE on the Activities of CAT, SOD, and GSH-Px in HepG2 Hepatoma Cells.** As shown in Figure 4, PPDE was found to reduce the activities of CAT, SOD, and GSH-Px in HepG2 hepatoma cells. As the PPDE concentration increased, the activities of CAT, SOD, and GSH-Px in the lysates of the treated cancer cells decreased significantly ( $P < 0.05$ ).

**3.5. Effects of PPDE on  $\gamma$ -GCS Activity and GSH Content in HepG2 Hepatoma Cells.** As shown in Figure 5, the activity of  $\gamma$ -GCS and the levels of GSH were highest in the untreated

HepG2 cells compared to both the untreated L02 cells and the HepG2 cells treated with PPDE. Specifically, after treatment of the HepG2 cells with PPDE (50, 100, and 200 µg/mL) for 24 h, the activity of  $\gamma$ -GCS and the levels of GSH in the HepG2 cells decreased gradually.

**3.6. Effects of PPDE on the mRNA Expression of Bax, Bcl-2, Caspase-3, Caspase-8, Caspase-9, SOD1, and SOD2 in HepG2 Cells.** The expression levels of several apoptosis-related genes were quantified by RT-qPCR. As shown in Figure 6, the expression levels of the genes encoding Bax, caspase-3, caspase-8, and caspase-9 in HepG2 cells were higher after treatment with 50, 100, and 200 µg/mL PPDE than in the untreated cells. Furthermore, the mRNA expression levels of Bcl-2, SOD1, and SOD2 were lower in the PPDE-treated HepG2 cells than in untreated cells. The expression levels of caspase-3, caspase-8, and caspase-9 genes

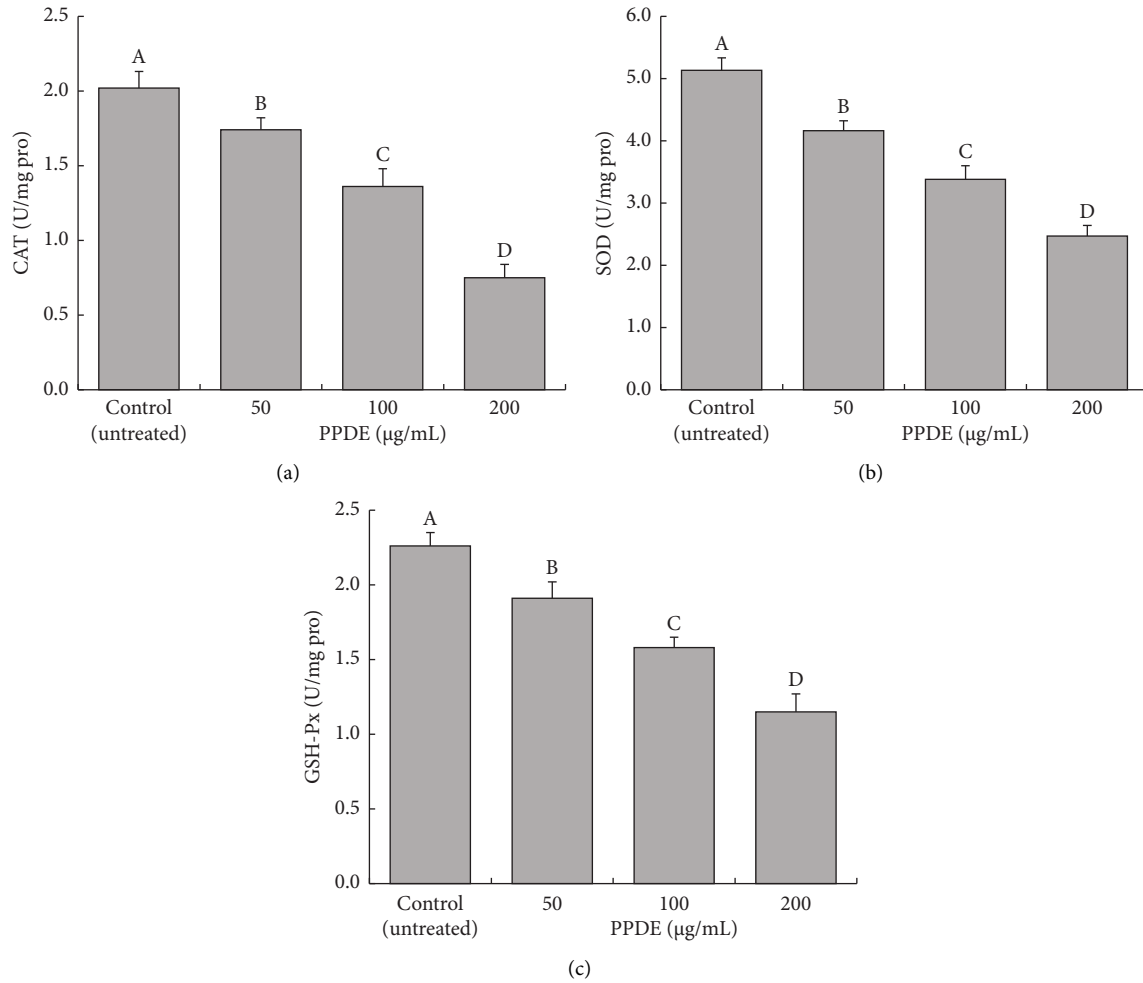


FIGURE 4: Effect of *Peucedanum praeruptorum* Dunn extract (PPDE) on the activities of CAT (a), SOD (b), and GSH-Px (c) in HepG2 human hepatoma cells ( $n = 8$ ). <sup>A-D</sup>Different lowercase letters indicate significant differences between the mean values of each group ( $P < 0.05$ ).

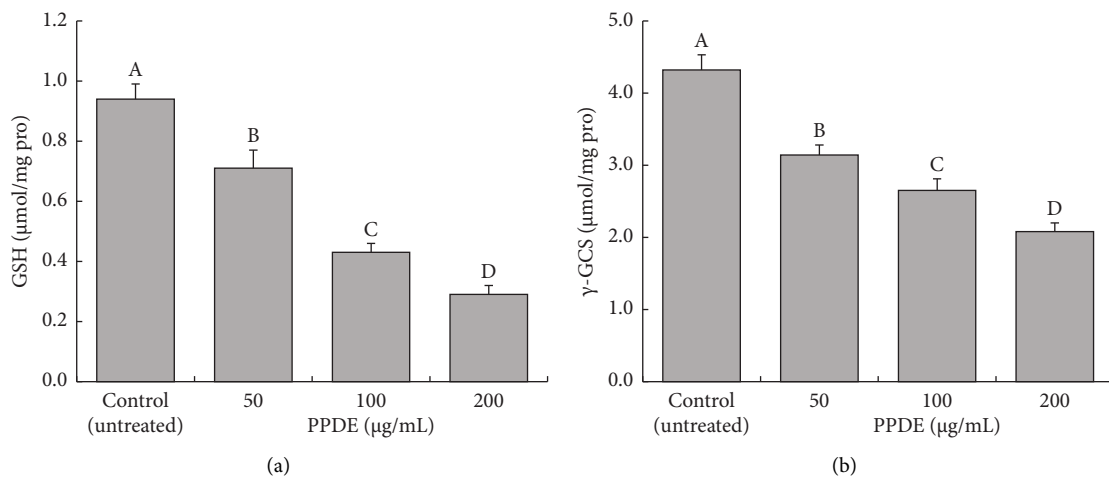


FIGURE 5: Effects of *Peucedanum praeruptorum* Dunn extract (PPDE) on the levels of GSH (a) and  $\gamma$ -GCS (b) in HepG2 human hepatoma cells ( $n = 8$ ). <sup>A-D</sup>Different lowercase letters indicate significant differences between the mean values of each group ( $P < 0.05$ ).

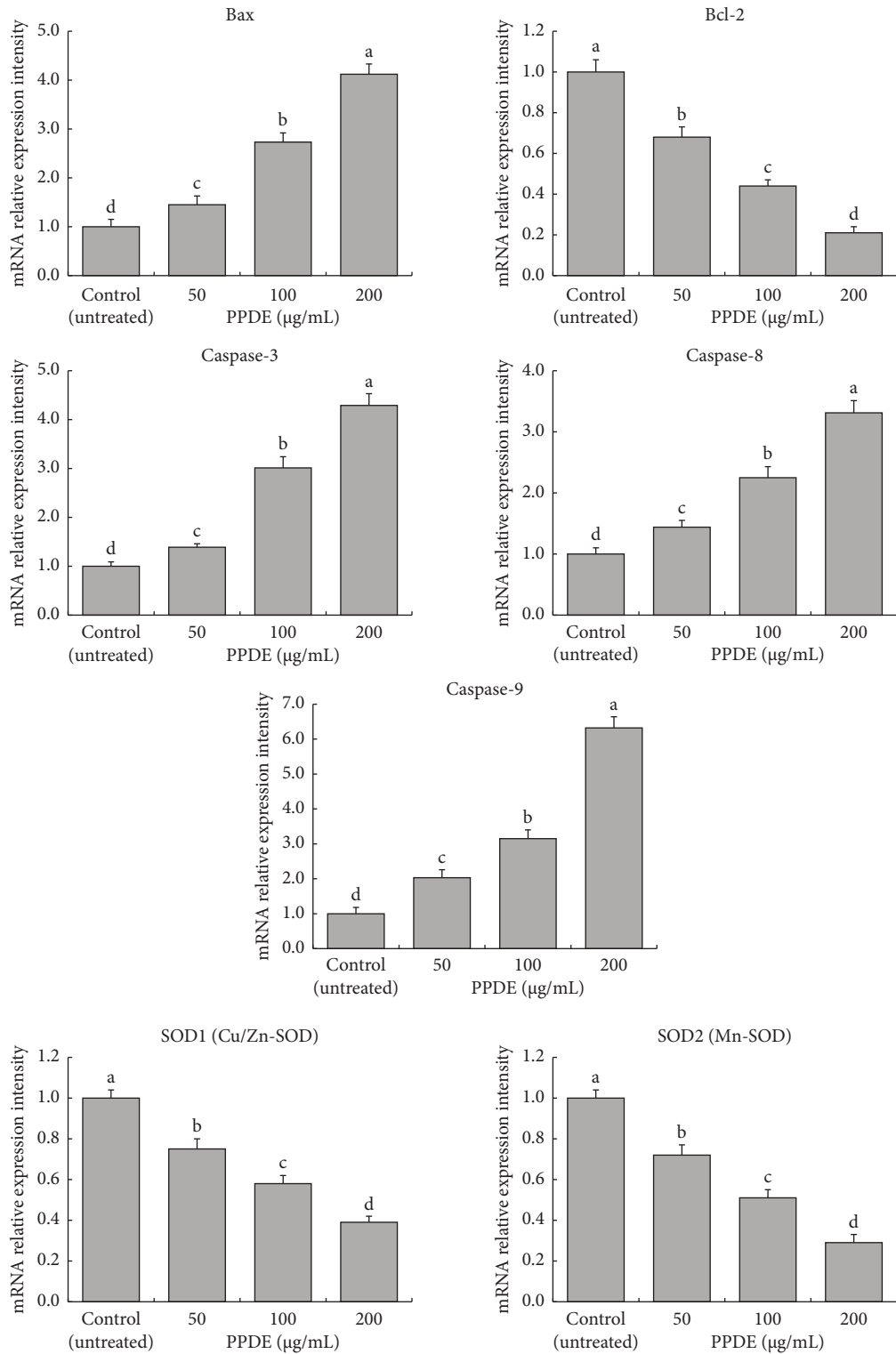


FIGURE 6: Effects of *Peucedanum praeruptorum* Dunn extract (PPDE) on mRNA expression of Bax, Bcl-2, caspase-3, caspase-8, caspase-9, SOD1, and SOD2 in HepG2 human hepatoma cells ( $n = 8$ ). <sup>a-d</sup>Different lowercase letters indicate significant differences between the mean values of each group ( $P < 0.05$ ).

were positively correlated with the concentration of PPDE, while the expression levels of SOD1 and SOD2 genes were negatively correlated with the concentration of PPDE.

#### 4. Discussion

Excessive generation or accumulation of reactive oxygen species (ROS) within cells can induce the peroxidation of unsaturated fatty acids in cell membranes. This process leads to an increased production of lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxynonenal, which have toxic effects on cells [19, 20]. Hence, MDA is commonly utilized as a marker to assess cell damage caused by oxidative stress [21]. In this study, we observed that incubation of HepG2 cells with PPDE resulted in higher levels of total ROS and MDA in the hepatoma cells compared to untreated Hep2 cells, suggesting that PPDE induced oxidative stress in these cells through the generation of ROS. Consequently, it was reasonable to predict that PPDE treatment would promote the death or inhibition of the proliferation of cancer cells, as a previous study demonstrated that *P. praeruptorum* Dunn had an inhibitory effect on the growth of cervical cancer cells *in vitro* [2]. Similar results were obtained in this study, in which PPDE was found to inhibit the proliferation of cultured HepG2 hepatoma cells.

Under normal physiological conditions, cancer cells are exposed to endogenous antioxidants such as enzymes (CAT, SOD, and GSH-Px) and the small molecule glutathione (GSH) that can induce oxidative stress, causing damage to cancer cells, in the presence of the immune system. SOD is an enzyme that converts  $O_2^-$  ions to  $H_2O_2$ , while CAT and GSH-Px break down  $H_2O_2$  into water [22]. In addition, GSH-Px utilizes GSH as a substrate to reduce  $H_2O_2$ , alkylhydroperoxides, and organic hydroperoxides (ROOH) into hydroxyl compounds (ROH) [23]. In this study, the activities of the main antioxidant enzymes (CAT, SOD, and GSH-Px), as well as the levels of  $\gamma$ -GCS and GSH, in HepG2 cells all decreased after treatment with PPDE. Reducing CAT and SOD activities would be expected to reduce the cells' ability to protect themselves against oxidative stress and increase lipid peroxidation, thereby promoting oxidative stress-related damage to cancer cells [24, 25].

As a significant nonenzymatic antioxidant in the body, GSH is involved in reducing toxic lipid peroxides and  $H_2O_2$  directly by means of GSH-Px. Moreover, it also indirectly inhibits free-radical chain reactions [26]. As the rate-limiting enzyme in GSH biosynthesis,  $\gamma$ -GCS plays a crucial role in regulating the intracellular levels of GSH. In this study, PPDE was found to decrease the levels of GSH and  $\gamma$ -GCS in HepG2 cells, thereby preventing the cancer cells from regulating intracellular oxidative stress. Therefore, the reduced viability and proliferation of the HepG2 cells after exposure to PPDE might be a result of the effect of PPDE on reducing the activities and levels of these antioxidant enzymes and small molecules. In addition, PPDE downregulated the mRNA and protein levels of SOD1 and SOD2 in HepG2 cells. By reducing the expression of SOD1 and SOD2 at the transcriptional level in cancer cells, PPDE decreased the overall SOD activity and consequently promoted oxidative stress and damage [24, 27].

Apoptosis was another mechanism underlying the cytotoxic effects of PPDE on cancer cells that was explored in this study. Apoptosis, or programmed cell death, is tightly regulated by the Bcl-2 family of proteins [28]. Bcl-2 prevents the release of cytochrome C from mitochondria and promotes cell proliferation [29]. Conversely, Bax, a typical apoptosis promoter, plays a crucial role in maintaining the balance of Bcl-2 in the body and influences the efficacy of cancer treatments [30]. Bcl-2 is expressed in lower quantities in early embryonic tissues, mature lymphocytes, epithelial cells, and neurons compared to cancer cells, wherein its expression is highly upregulated [31]. In contrast, Bax is widely distributed in normal tissues and cells throughout the human body, but it is expressed in very low quantities in cancer tissues. Consequently, the levels of Bcl-2 and Bax expression have been used as biomarkers for evaluating cancer prognosis [20, 29]. In our study, we found that PPDE significantly decreased Bcl-2 expression and increased Bax expression, potentially leading to the promotion of apoptosis in these cells.

Members of the caspase family of proteases, such as caspases 3, 8, and 9, play crucial roles in apoptosis as both mediators and executors of programmed cell death [32]. Caspase-8 and caspase-9 are upstream caspases in the apoptosis pathway that can activate downstream caspase-3 [33]. Caspase-3, in turn, is responsible for cleaving substrates that induce cell cycle arrest, inhibiting enzymes involved in cancer cell repair and breaking down the cancer cell system. In fact, most apoptosis processes, including those involving mitochondria, endoplasmic reticulum, and death receptors, require the active participation of caspase-3 [34]. Many of these processes are initiated and amplified by caspase-9 [35]. Caspase-8 can activate Bax, leading to lysis of the mitochondria and the release of Bcl-2, Bid, and cytochrome c. These released factors further activate caspase-9 and caspase-3 [36, 37]. Simultaneously, these factors integrate signals from both the death receptor and mitochondrial pathways, amplifying the apoptosis signal and promoting apoptosis [38]. In our discovery, we observed that PPDE increased the expression of caspase-3, -8, and -9 in HepG2 cells, highlighting an additional possible mechanism through which this extract exerted an anticancer effect *in vitro*.

Following HPLC analysis of the composition of the PPDE, caffeic acid, isochlorogenic acid C, myricetin, baicalin, luteolin, and kaempferol were the six active substances that comprised the extract. All of these compounds have demonstrated antioxidant effects, and several studies have shown that they inhibit the growth and viability of cancer cells *in vitro* [39–44]. Considering these findings, the potential of these substances to inhibit liver cancer cells *in vitro* was mentioned in the PPDE, presumably due to their combined effects.

#### 5. Conclusions

While PPDE inhibited the growth of HepG2 cells *in vitro*, it had no obvious toxic effect on L02 normal hepatocytes. Furthermore, PPDE promoted the apoptosis of HepG2 cells



in a dose-dependent manner, with the highest concentration (200 µg/mL) of PPDE demonstrating the highest rate of apoptosis. Further experimental results confirmed that PPDE reduced the viability and proliferation of HepG2 cells by inhibiting the expression and reducing the activities of antioxidant enzymes and small molecules that regulate intracellular oxidative stress. Taken together, these results demonstrated that PPDE is a biologically active plant extract with potential anticancer properties, warranting further scientific exploration into its clinical applications.

### Data Availability

The datasets generated for this study are available upon request to the corresponding author.

### Conflicts of Interest

All authors declare that they have no conflicts of interest.

### Authors' Contributions

Shiwen Hu and Pan Wang performed the majority of the experiments and wrote the manuscript. Yongchun Chen, Gang Kuang, Cun Wang, and Jing Luo contributed to the data analysis. Shaocheng Chen designed and supervised the study and checked the final manuscript. The authors Shiwen Hu and Pan Wang contributed equally to this work.

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