***Picrasma quassioides* Extract Elevates the Cervical Cancer Cell Apoptosis Through ROS-Mitochondrial Axis Activated p38 MAPK Signaling Pathway**

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Abstract. Background/Aim: *Picrasma quassioides* (*P. quassioides*) is used in traditional Asian medicine widely for the treatment of anemopyretic cold, eczema, nausea, loss of appetite, diabetes mellitus, hypertension etc. In this study we aimed to understand the effect of *P. quassioides* ethanol extract on SiHa cervical cancer cell apoptosis. Materials and Methods: The *P. quassioides* extract-induced apoptosis was analyzed using the MTT assay, fluorescence microscopy, flow cytometry and western blotting. Results: *P. quassioides* extract induced cellular apoptosis by increasing the accumulation of cellular and mitochondrial reactive oxygen species (ROS) levels and inhibiting ATP synthesis.

Pretreatment with *N*-Acetylcysteine (NAC), a classic antioxidant, decreased the intracellular ROS production and inhibited apoptosis. In addition, the P38 MAPK signaling pathway is a key in the apoptosis of SiHa cells induced by the *P. quassioides* extract. Conclusion: The *P. quassioides* extract exerts its anti-cancer properties on SiHa cells through ROS-mitochondria axis and P38 MAPK signaling. Our data provide a new insight for *P. quassioides* as a therapeutic strategy for cervical cancer treatment.

Cervical cancer is the fourth most common cancer affecting women worldwide (1), putting a huge financial and medical burden on society, so it is necessary to work together globally to improve its treatment. At present, the methods of treating cervical cancer mainly include surgery, chemotherapy and adjuvant chemotherapy (2, 3), with certain side effects, such as loss of appetite and weight loss. Screening of natural products from plant sources, such as herb extracts, with minimal side effects that can be obtained in a cost-effective manner have received global attention for treating several types of cancers (4-6).

Picrasma quassioides (*P. quassioides*) is a Asian traditional medicine mainly distributed in Korea and South China (7). The bark, roots, stems and leaves are traditionally used for the treatment of anemopyretic cold, sore throat, dysentery, eczema, nausea, loss of appetite, diabetes mellitus, hypertension and other conditions (8-10). Chemical investigation has shown that alkaloids, including β -carboline (11, 12), canthinone (13) and bis β -carboline alkaloids (14,

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15) are the principal active components in *P. quassioides*. These active materials show anti-inflammatory, anti-hypertensive and anti-angiogenic properties in humans (8, 9). At the same time, recent studies have shown that the monomers extracted from *P. quassioides* have the ability to increase intracellular reactive oxygen species and induce apoptosis in HepG2 and Hep3B liver cancer cells (16, 17). Despite these data, the anti-cancer effect of *P. quassioides* on cervical cancer and the possible underlying mechanism has not yet been studied.

Reactive oxygen species (ROS) formed from various cellular metabolites play a vital role in many biological processes, including oxygen sensing, cell differentiation and adaptive immunity (18). Disturbances in the balance between intracellular antioxidants and ROS can lead to the dysfunction of mitochondria (19), which can promote cell and tissue oxidative stress *in vivo* and *in vitro*. Most active radicals are considered as natural by-products of mitochondrial activation. Generally, low levels of ROS can serve as signal molecules to regulate numerous physiological processes, while excess levels can lead to caspase activation and cytochrome c release and promote cell death (20), causing various diseases, such as cardiovascular disease, diabetes, and cancer (21, 22). Compared to normal cells, cancer cells have higher ROS levels. High levels of ROS change the redox state of the cell itself, promoting the occurrence and development of malignant phenotypes as reflected in the continuous proliferation of cancer cells (20).

The biochemical characteristics of cancer cells make them more vulnerable to further ROS increase induced by exogenous ROS-generating agents (pro-oxidants) compared to normal cells (23). Based on these facts, new anti-cancer strategies for ROS have been developed to selectively kill cancer cells by promoting excessive ROS production and inducing cancer cell mitochondrial dysfunction (24-26).

Herein, the effect of *P. quassioides* extraction on the survival of cervical cancer was evaluated by examining the viability, apoptosis and the changes in apoptosis-related protein expression in SiHa cervical cancer cells. We also investigated the effect of *P. quassioides* extract on SiHa cell migration, colony formation, mitochondrial membrane potential, cellular and mitochondrial ROS levels and ROS-dependent signaling pathways, such as MAPK signaling. Our findings may give a new sight in understanding the potential anti-cancer properties of *P. quassioides* extract on cervical cancer.

Materials and Methods

Extract preparation. A crude extract of *Picrasma quassioides* was prepared essentially as described before (27). Briefly, air-dried stems of *Picrasma quassioides* (50 g) were soaked in 500 ml of ethyl alcohol, and then extracted by shaking it at 200 rpm for 5 h

at 37°C. The filtrate was recovered, and an additional 500 ml of ethyl alcohol were used for filtering the residue. The process of recovery and filtration of the extract was repeated three times and it was concentrated on a rotary evaporator for 12 h at 55°C. The concentrated extract was labeled as PQ and stored in a refrigerator at 4°C until further use.

Cell culture. SiHa cervical cancer and QSG-7701 hepatocyte normal cells were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) medium supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), penicillin (100 U/ml) and streptomycin (100 mg/ml) (P/S) (Solarbio life sciences, Beijing, PR China), and were incubated at 37°C with 5% CO₂.

Cell viability assay. The cell viability was analyzed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide, Sigma-Aldrich, St. Louis, MO, USA] assay and CCK-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl) -5-(2,4-isulphophenyl)-2H-tetrazolium, monosodium salt, cell counting Kit-8, MedChem Express, Shanghai, PR China]. CCK-8 is more sensitive than MTT, but there is no significant difference between them in detecting cell viability. The SiHa and QSG-7701 cells were seeded in 96-well plates at 4×10³ cells/well and treated with PQ for 24 h. Then, 10 µl (0.5 mg/ml) of MTT or 10 µl of the CCK-8 were added to each well and incubated for 2 h at 37°C in 5% CO₂. Subsequently, the supernatant was removed and formazan was solubilized with dimethyl sulfoxide. Absorbance was measured at 490 nm or 450nm using a UV MAX kinetic microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Apoptosis detection by Annexin V-FITC. The SiHa cells were seeded in 6-well plates at 2×10⁵ cells/well following a 24 h incubation with PQ treatment. To detect apoptosis, cells were prepared using the Annexin V-FITC and propidium iodide (PI) detection Kit (Solarbio life sciences, Beijing, PR China), according to the manufacturer's protocol and were analyzed by fluorescence microscopy (EVOS®xl core cell culture microscope, Advanced Microscopy Group, Paisley, Scotland, UK) and flow cytometry (FACSCalibur, BD Biosciences, Franklin Lakes, NJ, USA). The results were analyzed using the WinMDI (Version 2.9, BD Biosciences) software.

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Figure 1. Effect of PQ on cell viability, apoptosis, cell migration and colony formation in SiHa cells. (A) Cell viability of SiHa cervical cancer cells and QSG-7701 normal liver cells were measured by MTT assay following treatment with PQ (0, 20, 30, 40, 50, 60 µg/ml) and 5-FU (60 µg/ml). 5-FU caused a decrease in cell viability in both SiHa and QSG-7701 cells, whereas PQ only affected the viability of SiHa cancer cells. (B) Annexin V-FITC/PI staining and flow cytometry (left side graphs) and increased levels of SiHa cells apoptosis after PQ treatment quantified (right side graph). (C) Fluorescence and light microscopy images of SiHa cells stained with Annexin-V and PI showed increased apoptosis and levels of dead cells with increasing concentration of PQ, respectively. (D) Wound healing assay (left panel) and colony formation assay (right panel) of SiHa cells showing their decreased migration capacity after PQ treatment (scale bar=100 µM). Data are presented as the mean±standard error of the mean from three different samples. *p<0.05, **p<0.01, ***p<0.001.

Figure 2. Effect of PQ on cellular and mitochondrial ROS, mitochondrial membrane permeability and ATP content in SiHa cells. (A) Dihydroethidium (DHE) and Hoechst staining of SiHa cells after treatment with increasing concentrations of PQ showed increased cellular reactive oxygen species (ROS) levels, (B) MitoSOX and Hoechst staining show increased mitochondrial ROS in cervical cancer cells, and (C) increased JC-1 staining showed an increase in the mitochondrial membrane potential. (D) The ATP levels in SiHa cells decreased following PQ treatment. Data are presented as the mean standard error of the mean of three different samples. * $p < 0.05$.

Wound healing assay. SiHa cells were seeded in 6-well plates at 1×10^6 cells/well. A linear scratch was made by introducing a small pipette tip in the cell monolayer. The cells were rinsed with $1 \times \text{PBS}$ three times and PQ (60 $\mu\text{g/ml}$) was added. Photomicrographs of the migrated cells were taken at 0 and 24 h using a fluorescence microscope (EVOS[®]x1 core cell culture microscope).

Colony-formation assay. SiHa cells (1×10^3 cells/well) were seeded in six-well plates, treated with PQ (60 $\mu\text{g/ml}$) and maintained at 37°C in a 5% CO_2 incubator for 10 days. The cells were then washed with $1 \times \text{PBS}$, fixed for 10 min with 3.7% formaldehyde, treated for 20 min with methanol, and stained for 30 min with 0.05% crystal violet. The plates were washed with $1 \times \text{PBS}$ three times before image capturing.

Detection of ROS production. Cells were seeded in 6-well plates at 2×10^5 cells/well and treated with PQ for 24 h. The changes in cellular and mitochondrial ROS levels were determined using Dihydroethidium (DHE, Beyotime Biotechnology, Shanghai, P.R. China), which is specific for superoxide and hydrogen peroxide in living cells and MitoSOX (Thermo Fisher Scientific, Waltham, MA, USA) staining, which detects superoxide in the mitochondria of living cells. Nuclei were visualized using the Hoechst 32258 (Thermo Fisher Scientific, Waltham, MA, USA) staining. Nuclear staining was observed qualitatively under a microscope after 20 min of incubation with the Hoechst dye.

Mitochondrial depolarization assay. Changes in the levels of the cell mitochondrial membrane potential in SiHa cells treated with PQ

Figure 3. Effect of PQ on the expression of apoptosis and apoptosis-related proteins as well as MAPK signaling pathway activation in SiHa cells. Western blotting shows (A) increased expression of pro-apoptotic c-cas3, c-cas9, Bax and decreased expression of the anti-apoptotic Bcl-x_L. (B) PQ treatment also increased the phosphorylated proteins p-38, ERK and JNK, although p-ERK levels returned to normal after a 24 h incubation with PQ. (C) PQ induces apoptosis in SiHa cells and the use of the SB203580 p38 inhibitor repressed the PQ-induced cellular apoptosis, whereas the inhibitors of ERK (PD98059) and JNK (SP600125) did not. Quantification of these data are presented by the mean±SD of PQ-treated cells with and without the different inhibitors from three different experiments. **p*<0.05, ***p*<0.01, ****p*<0.001.

were determined using JC-1 (Beyotime, Shanghai, PR China). Cells were incubated with 20 mM JC-1 for 15 min at 37°C and then washed with PBS. After washing with PBS, images were taken using a fluorescence core cell culture microscope (EVOS[®]x) and the fluorescence intensities were observed qualitatively.

Intracellular ATP determination. Cells were seeded in 6-well plates at 2×10^5 cells/well and treated with PQ for 24 h. Adenosine triphosphate (ATP) were determined using the ATP determination Kit (Thermo Fisher Scientific, Waltham, MA, USA). Luminescence

(RLU) was immediately measured using a fluorescent chemical analyzer (Infinite 200 PRO Microplate Reader, Tecan Trading AG, Männedorf, Switzerland) according to the manufacturer's protocol.

Western blot analysis. Cell protein lysates were separated in 12% sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). The membranes were blotted with primary antibodies against caspase3 (Cell Signaling Technology, Beverly, MA, USA, #9661), caspase-9 (Cell Signaling Technology, #9505), Bax (Abcam Cambridge, MA,

Figure 4. The regulatory function of PQ-induced mitochondria dysfunction is dependent on cellular ROS levels. Cell pre-treated with N-Acetylcysteine (NAC) followed by treatment with PQ showed (A) reduced cellular and (B) mitochondrial ROS levels as well as (C) the mitochondrial membrane potential in SiHa cells. (D) On the contrary, NAC treatment significantly restored the PQ-induced inhibition of ATP synthesis. Data are presented as the mean $\pm$ standard error of the mean from three different experiments. * $p < 0.05$.

USA, #32503), Bcl-x_L (Santa Cruz Biotechnology, Dallas, TX, USA, #sc-8392), ERK (Santa Cruz Biotechnology, #sc-135900), p-ERK (Santa Cruz Biotechnology, #sc-7383), P38 (AbFrontier, Seoul, Republic of Korea, #LF-MA0126), p-P38 (Santa Cruz Biotechnology, #sc-7973), JNK (Santa Cruz Biotechnology, #sc-7345), p-JNK (Santa Cruz Biotechnology, #sc-6254), α -tubulin (Abcam Cambridge, #ab7291) (dilution 1:5,000) at 4°C overnight. The membranes were washed five times with Tris buffered saline containing Tween-20 (TBST) [10 mM Tris HCl (pH 7.5), 150 mM NaCl and 0.2% Tween-20] and were subsequently incubated with Horseradish Peroxidase-conjugated goat anti-rabbit IgG (Sangon Biotech, Shanghai, PR China) or anti-mouse IgG (Sangon Biotech) for 1 h at room temperature (RT). After the removal of excess antibodies by washing with TBST, specific binding was detected

using a chemiluminescence detection system (GE Healthcare Life Sciences, Chalfont, UK), according to the manufacturer's protocol.

Statistical analysis. The data are depicted as mean $\pm$ SEM values. Student's *t*-tests were performed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA), and values of $p < 0.05$ were considered indicative of significant difference.

Results

PQ treatment increased the cellular apoptosis, cell migration and colony formation in SiHa cervical cancer cells. To investigate effect of PQ on cellular cytotoxicity, the SiHa

Figure 5. Effect of NAC treatment on cell viability, apoptosis, cell migration, colony formation and apoptosis-related protein expression in SiHa cells. (A) NAC treatment restored the PQ-induced decrease in cell viability, and (B, C) decreased cell apoptosis as shown by flow cytometry and fluorescence microscopy, respectively. (D) Wound healing assay and colony formation assay showed a restoration in the levels of migratory and proliferative ability of PQ- treated cervical cancer cells when these were pre-treated with NAC. (E) NAC pretreatment decreased the expression levels of pro-apoptotic c-cas3 and c-cas9, as well as the anti-apoptotic Bcl-x_L, as detected by Western blot. (F) WB data are presented as the mean±standard error of the mean from three different experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

cervical cancer and QSG-7701 normal liver cells (used as an experimental control) were treated with 5-FU (60 µg/ml) and PQ in various concentrations (0, 20, 30, 40, 50 and 60 µg/ml) for 24 h before the MTT assay. The results showed that both 5-FU and PQ have strong cytotoxic effects on SiHa cervical cancer cells with higher levels exhibited by PQ compared to the 5-FU treatment, while both of them have a little cytotoxic effect on QSG-7701 normal liver cells (Figure 1A). To determine whether PQ can induce cell apoptosis, the SiHa cells were treated with PQ (0, 40 and 60 µg/ml) for indicated duration times (0, 6, 12 and 24 h) and were stained using the Annexin-V FITC and PI detection Kit. The apoptotic cells using recombinant Annexin V conjugated to green-fluorescent FITC dye, PI stains necrotic cells with red fluorescence. After treatment with both probes, apoptotic cells show green fluorescence, dead cells show both red and green fluorescence, and live cells show little or no fluorescence. The results revealed that PQ treatment can significantly increase the cellular apoptosis in a time- and dose-dependent manner in SiHa cervical cancer cells (Figures 1B and C). Furthermore, as shown in Figure 1D, PQ treatment significantly inhibited the cell migration and colony formation in SiHa cervical cancer cells.

PQ treatment accelerates the cellular and mitochondria ROS levels and mitochondrial dysfunction. In order to detect whether PQ can increase intracellular ROS, SiHa cervical cancer cells were treated with PQ in various concentrations (0, 40, 60 µg/ml) for 24 h and the cellular ROS levels were detected by DHE & Hoechst staining. The results showed that PQ can significantly increase the intracellular ROS contents in SiHa cells (Figure 2A). At the same time, MitoSOX (a mitochondrial ROS indicator, which can be oxidized by superoxide in live cells) & Hoechst staining showed that PQ can also effectively increase ROS levels specifically in mitochondria (Figure 2B). Higher mitochondria ROS levels will cause the disruption of mitochondrial function. In order to determine whether PQ-induced ROS leads to oxidative stress that can cause a shift in the redox balance of mitochondria, we examined the increase of mitochondrial permeability in SiHa cells using the permeability of JC1 as a readout. JC-1 is a membrane permeable dye that selectively enters the mitochondria and can detect the intracellular mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-1 accumulates inside the mitochondria forming aggregates, which can generate red fluorescence; when the mitochondrial membrane potential is low, JC-1 cannot be gathered in the matrix of the mitochondria, it remains a monomer and produces green fluorescence (28). The results showed that the ratio of green fluorescence (low $\Delta\Psi_m$) to red fluorescence (high $\Delta\Psi_m$) of JC-1 in SiHa cells was significantly increased in various concentrations (0, 40, 60 µg/ml) of PQ treatment (Figure 2C).

In addition to mitochondrial membrane potential, ATP-synthesis ability is also a fundamental indicator evaluating mitochondrial function (29). Therefore, we examined the intracellular ATP contents of SiHa cells treated with different concentrations (0, 40, 60 µg/ml) of PQ for 24 h. The results showed that PQ treatment significantly inhibited the ATP synthesis in SiHa cells (Figure 2D).

PQ induces mitochondria-dependent apoptosis and MAPK signaling pathways in SiHa cells. In order to detect whether PQ is responsible for mitochondrial-dependent apoptosis, we treated SiHa cells with PQ (60 µg/ml) for the indicated duration times (0, 1, 3, 6, 12 and 24 h), and we then measured the protein levels of different caspases and their upstream B-cell lymphoma-2 (Bcl-2) family proteins. The results showed that the expression levels of cleaved-caspase3, cleaved-caspase9 and Bax proteins were up-regulated in PQ-treated SiHa cells, while the Bcl-x_L proteins were down-regulated following PQ treatment (Figure 3A).

MAPK family members, including c-Jun N-terminal kinase (JNK), ERK1/2, and P38 MAPK, are involved in apoptosis (30). Following the same treatment as above, we assessed protein expression level changes of the MAPK signaling pathway. Our results showed that PQ treatment significantly increased the p-P38, p-ERK and p-JNK protein expression levels in SiHa cells (Figure 3B). To understand the role of MAPK in PQ-induced cell apoptosis, the SiHa cells were tested using Annexin-V-FITC/PI staining after they were pre-treated with inhibitors of MAPK, such as i) SB203580 (P38 inhibitor, 50 nM), ii) PD98059 (ERK inhibitor, 10 µM) and iii) SP600125 (JNK inhibitor, 40 nM) for 30 min followed by treatment with PQ for 24 h. The results showed that suppressing P38 with SB203580 significantly decreased the PQ-induced cellular apoptosis in SiHa cells but not ERK or JNK inhibitors (Figures 3C and D).

Inhibition of intracellular ROS reduces mitochondrial damage, cellular apoptosis, cell migration and colony formation in SiHa cells. To detect whether ROS is a key molecule mediating PQ-induced apoptosis, the SiHa cells were pre-treated with N-Acetylcysteine (NAC, ROS inhibitor, 5 mM) for 30 min followed by treatment with PQ (60 µg/ml) for 24 h. The cellular and mitochondrial ROS levels as well as the mitochondrial membrane potential were detected using DHE, MitoSOX and JC-1 staining, respectively, and fluorescence microscopy. The results showed that treatment with NAC significantly reduced the PQ-stimulated cellular and mitochondrial ROS levels as well as the mitochondrial membrane potential in SiHa cells (Figures 4A-C). Furthermore, as shown in Figure 4D, NAC treatment significantly restored the PQ-induced inhibition of ATP synthesis. We also examined the effect of NAC on PQ with regard to the cell viability, apoptosis, migration, proliferation,

as well as apoptosis-related protein expression using CCK-8 assay, Annexin-V staining, wound healing assay, colony forming assay and western blot in SiHa cervical cells, respectively. The results showed that scavenging of ROS with NAC treatment significantly restored the PQ-induced increase in cytotoxicity and apoptosis (Figures 5A-C) in SiHa cells, and also recovered the inhibition of cell migration and proliferation (Figure 5D). Western blot analysis showed that NAC could dramatically restore the PQ-induced up-regulation of pro-apoptotic c-cas3 and c-cas9 and down-regulation of anti-apoptotic Bcl-xL proteins expression (Figure 5E).

Discussion

Cancer is a complex process involving a series of proliferative signals, including escape from growth inhibition, resistance to cell death, and uncontrolled proliferation as well as increased invasion and metastasis of cancer cells. A great focus has been on the development of new anti-tumor drugs, including natural plant drugs and their derivatives (6, 31). In fact, herbal medicinal have been tested as potential treatment modalities for a variety of cancers, including leukemia, ovarian, testicular, lung, liver, esophageal, stomach, colon, and rectum cancer (32-34). In the present study, we reported that the *P. quassioides* extract bears anti-cancer properties in SiHa cervical cancer cells with a low cytotoxicity in normal cells, promoting apoptosis and inhibiting cancer cell migration and proliferation.

ROS, known as second messenger, play pivotal roles in cell survival, proliferation, migration and apoptosis (35). It was well known that mitochondria are not only the main source of intracellular ROS production but are considered as the main targets of ROS damage (36, 37). In pathological conditions, excessive ROS formation in mitochondria can reduce energy synthesis, such as in the form of ATP, and accelerate the production of additional ROS (25, 38), accumulation of which can damage the mitochondrial membrane and potentially lead to mitochondria-dependent apoptosis in cells (20, 39, 40).

In this study, our experimental data revealed that PQ treatment significantly increased intracellular and mitochondrial ROS production, as well as ATP synthesis accompanied by a decrease in the mitochondrial membrane potential in SiHa cervical cancer cells. Members of the caspase and Bcl-2 families are involved in promoting mitochondria-dependent cell apoptosis (41, 42). In our research, we found that PQ treatment significantly up-regulated the pro-apoptotic proteins, such as cleaved-caspase9, cleaved-caspase3 and Bax expression levels in SiHa cervical cancer cells while down-regulated the anti-apoptotic protein, Bcl-x_L. Furthermore, ROS inhibition significantly attenuated apoptosis as well as their migration, proliferation and mitochondrial permeability in PQ-treated SiHa cervical cancer cells. All of these findings strongly suggest that ROS is a key

point in PQ stimulation of normal function in SiHa cervical cancer cells. Despite, this understanding, the role of PQ on the mitochondrial membrane potential as well as the molecular mechanisms responsible for the increase of cellular ROS levels remains to be addressed in future studies.

Downstream of ROS, the MAPK signaling is involved in many cellular responses, such as senescence, proliferation, differentiation, and apoptosis (41, 43). Members of the MAPK signaling involve the P38, ERK and JNK, whose pathways have been linked to apoptosis in various cancer cells as well as in cervical cancer (44, 45). Recently, it was reported that a Brevilin A induces apoptosis in Breast Cancer Cells through the activation the JNK and P38/MAPK (46), while inhibition of the ERK-MAPK efficiently prevented apoptosis in hepatocellular carcinoma cells (47). In the present study, we also reported that PQ treatment significantly up-regulated the phosphorylation of P38, suggesting that MAPK signaling is involved in the PQ-stimulated cell apoptosis in SiHa cervical cancer cells.

There are some limitations in this study. First, the extract from *P. quassioides* is not a compound, thus we can't ensure the correct component that is responsible for activating apoptosis in cells, and second, we have no direct evidence to understand the molecular mechanisms linking ROS, P38/MAPK and mitochondrial dysfunction. Further studies will give answers to these questions.

In conclusion, our results revealed that *P. quassioides* extract exerts anti-cancer properties by increasing cytotoxicity and apoptosis, and inhibiting migration and proliferation in SiHa cervical cancer cells. The disruption of these cellular functions was caused by the accumulation of intracellular and mitochondrial ROS which in turn activated the P38/MAPK and the mitochondria/dependent apoptosis signaling pathway. Our findings provide insight for *P. quassioides* as a potential therapeutic strategy for the treatment of cervical cancer.

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Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

YXG, YL, YHJ, TK, YDC and HNS performed the construct of the model and wrote the manuscript. YHH, JL, GNS, DPX, CXR, LYY,

DSL, JSK, YJJ, and JK performed the data analysis, JL and YHP provided *P. quassioides* extract reagents, TK, YDC and HNS were substantial contributors to study conception and design. All authors read and approved the final manuscript.

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