

ORIGINAL ARTICLE

Berberine inhibits human gastric cancer cell growth via deactivation of p38/JNK pathway, induction of mitochondrial-mediated apoptosis, caspase activation and NF- κ B inhibition

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Summary

Purpose: Gastric cancer accounts for considerable mortality across the globe. In this study the anticancer effects of a natural compound Berberine were investigated in vitro. Effects of berberine on cell migration, cellular apoptosis, Nf- κ B and JNK/p38 signalling pathways were also studied.

Methods: The cell viability of SNU-1 gastric cancer cells after berberine treatment was evaluated by CCK-8 assay, while the effects on cell migration were checked by wound healing assay. Effects on cellular apoptosis were evaluated by fluorescence microscopy using DAPI staining, as well as using flow cytometry with annexin V/propidium iodide (PI) staining. Effects on apoptosis-related protein expressions were checked by western blot method.

Results: The results showed that Berberine decreased the viability of the gastric cancer SNU-1 cells and exhibited an IC_{50} of 30 μ M. The cytotoxicity of Berberine was also inves-

tigated on the normal GES-1 gastric cells and it was found that Berberine exerted very low toxic effects on these cells and exhibited an IC_{50} of 120 μ M. Berberine also caused remarkable changes in the morphology of the SNU-1 cells. PI and DAPI staining revealed that Berberine prompted apoptosis of the SNU-1 cells in a dose-dependent manner. The apoptotic cells increased from 2.2% in control to around 35% at 30 μ M concentration. Berberine also suppressed the migration and invasion of the gastric cancer cells via blocking of the JNK/p38 signalling pathway.

Conclusions: Berberine may act as a promising drug candidate for gastric cancer as demonstrated from the current study.

Key words: apoptosis, gastric cancer, invasion, migration, cell cycle arrest, berberine

Introduction

Over the last few decades, utilization of plant extracts or plant-derived drugs has gained tremendous attention owing to the lower side effects and higher potency [1]. Many of the currently used drugs originate from plants and several of the synthetic drugs are actually the derivatives of their natural counterparts. Moreover great amount of research is going on for establishing the pharmacological correlation with ayurvedic/alternative medicines [2]. Berberine is an important plant secondary metabolite belonging to the class

of metabolites known as isoquinoline alkaloids. It has been isolated from the different plant parts of various plant species *Berberis vulgaris* (barberry) [3]. A number of studies have been carried out to evaluate the anticancer activity of Berberine which have shown that its anticancer activities are due to multiple mechanisms [4]. This study was designed to investigate anticancer effects against the human gastric cancer cells. Gastric cancer (GC) is one of the prevalent types of cancers and more than 50% of all the cancers detected in East Asian countries

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are GCs [5]. Despite the decline in the frequency of GC, it is still reported to be one of the commonly diagnosed cancers across the world [6]. The treatment for GC generally involves surgery and chemotherapy but the clinical outcomes are still very poor. Besides, the adverse effects of the anti-cancer agents used for the treatment of GC affect the overall health of the patients [7]. Herein we examined the anticancer effects of Berberine, an important triterpenoid of plant origin, against the human SNU-1 gastric cancer cells and attempted to explore the molecular mechanisms responsible for its anticancer effects.

Methods

Cell viability determination

In brief, the SNU-1 gastric cancer and normal GES-1 cells were seeded in 96-well plates and subjected to treatment with varied concentrations of Berberine at 37°C for 24 h. Thereafter 10 μ L of CCK-8 solution were added to the cell culture and incubated for 2 h at 37°C in an incubator (5% CO₂, 95% O₂). OD₄₅₀ was taken with the help of a microplate reader to determine the cell viability.

Detection of apoptosis

The SNU-1 cells (0.6 $\times 10^6$) were seeded in 6-well plates and subjected to incubation with varied concentration of Berberine for 24 h at 37°C. As the cells cast off 10 μ L cell culture were put onto glass slides and stained with DAPI. The slides were cover-slipped and examined with a fluorescent microscope.

Annexin V/PI staining

ApoScan kit was used to determine the apoptotic SNU-1 cell percentage. In brief, Berberine-treated SNU-1 cells (5 $\times 10^5$ cells per well) were incubated for 24 h. This was followed by staining of these cells with annexin V-FITC/PI. The percentage of apoptotic SNU-1 cells at each concentration was then determined by flow cytometry.

Wound healing assay

In brief, SNU-1 cells were cultured in 6-well plates for 24 h. Then, after making a scratch line on the cells using a 200 μ L sterile pipette tip, the plates were incubated at 37°C in 5% CO₂. Wound healing was observed at 0 and 24 h using an inverted microscope system.

Western blot analysis

Protein expression estimation was carried out by western blotting. The Berberine-treated SNU-1 gastric cancer cells were lysed in RIPA lysis buffer containing protease inhibitor for collection of protein extracts. From each group equal amounts of protein extracts were run on SDS-PAGE, followed by transferring to polyvinylidene fluoride (PVDF) membrane. Next, fat-free milk was used to block the membrane at room temperature for 1 h. Afterwards, the membranes were treated with primary antibodies at 4°C overnight. Subsequently, the membranes were incubated with secondary antibodies. Finally the band signal was detected by Odyssey Infrared Imaging System. Actin was used as control for normalisation.

Statistics

The results are presented as mean \pm standard deviation from three independent experiments. Differences between the groups were examined by Student's *t*-test using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). *P* < 0.05 was considered to indicate a statistically significant difference.

Results

Berberine suppresses the growth of SNU-1 cells via deactivation of p38/JNK pathway

To ascertain the growth inhibitory effects of Berberine, the SNU-1 cells were treated with 0–200 μ M of Berberine and then subjected to CCK8 assay. The results of the CCK8 cell viability assay revealed that Berberine caused concentration-dependent decrease in the viability of the SNU-1 cells (Figure 1A). It was further found that at 24 h of incubation, Berberine showed an IC₅₀ of 25 μ M against the

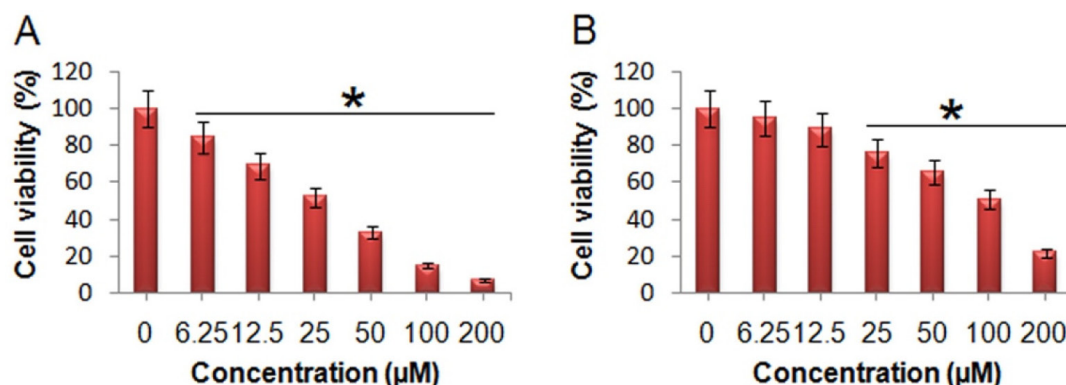


Figure 1. Effect of berberine on the viability of (A) gastric cancer SNU-1 and (B) GES-1 non-cancerous cells as depicted by CCK-8 assay. The experiments were performed in triplicate and shown as mean \pm SD (**p* < 0.01).

SNU-1 gastric cancer cells. However, the molecule did not exhibit significant toxic effects on the normal human gastric GES-1 cells as evidenced from the IC_{50} of more than 90 μ M (Figure 1B). Next, we sought to know if Berberine has any effect on the p38/JNK signalling pathway, so western blot of the SNU-1 cells treated with at 0, 12.5, 25 and 50 μ M of Berberine was performed. The results showed that Berberine caused decrease in the expression of p-p38 and p-JNK in a concentration-dependent manner (Figure 2). However, no visible effects were observed on the total JNK and p38.

Berberine induces apoptosis in SNU-1 cells

The apoptosis in the Berberine-treated SNU-1 cells was assessed by DAPI staining. The DAPI staining revealed that the molecule triggered apo-

ptosis as evidenced from nuclear fragmentation of the Berberine-treated SNU-1 cells (Figure 3). Moreover, the results of the DAPI-positive cells increased with increase in the concentration of Berberine, indicative of apoptotic cell death. Annexin V/PI staining showed that the apoptotic SNU-1 cell percentage increased to about 29.51% at 50 μ M concentration of Berberine as compared to approximately 1.82% in the untreated SNU-1 cells (Figure 4). Additionally, the western blotting analysis showed that Berberine caused increase in Bax and decrease in Bcl-2 expression in a concentration-dependent manner (Figure 5).

Berberine causes activation of caspases in SNU-1 cells

The effect of Berberine were also examined on the expression of caspases at concentrations of 0,

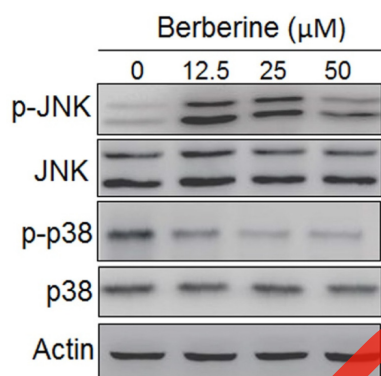


Figure 2. Effect of berberine on the p38/JNK signalling pathway as depicted by western blot analysis. The experiments were performed in triplicate.

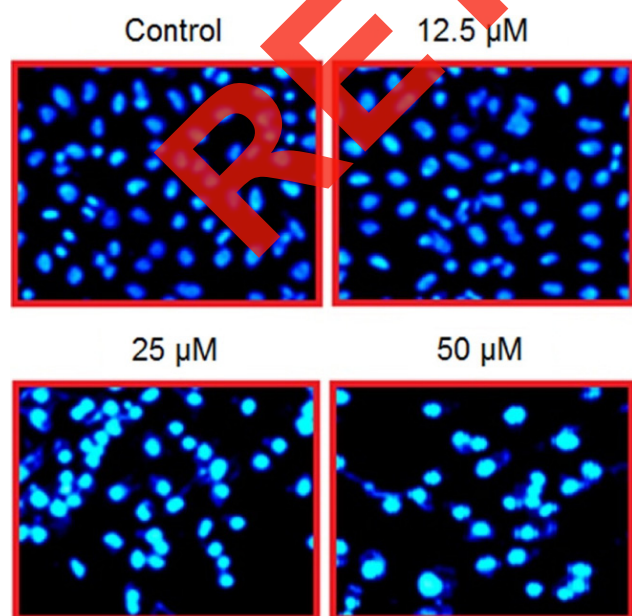


Figure 3. DAPI staining showing the induction of apoptosis in SNU-1 cells at indicated concentrations. The experiments were performed in triplicate.

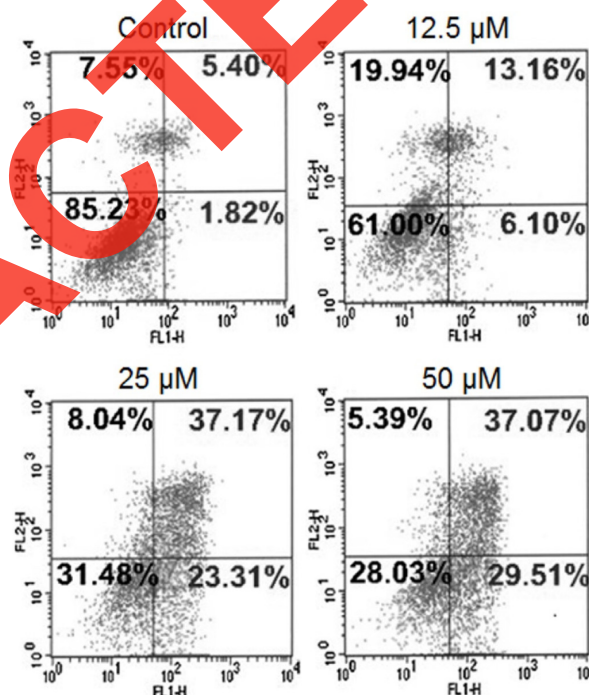


Figure 4. Annexin V/PI staining showing the percentage of apoptotic SNU-1 cells at indicated concentrations. The experiments were performed in triplicate.

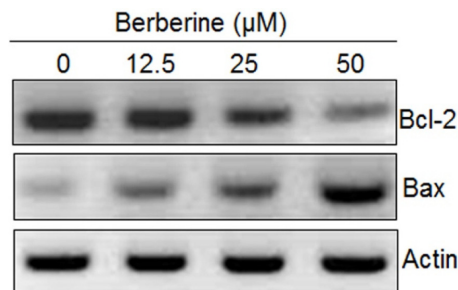


Figure 5. Effect of berberine on the expression of Bax and Bcl-2 in SNU-1 gastric cancer cells as depicted by western blot analysis. The experiments were performed in triplicate.

12.5, 25 and 50 μM by western blot analysis. It was found that the expression of all the caspases ie., the caspase-3, caspase-8 and caspase-9 increased upon treatment of the SNU-1 gastric cancer cells by this molecule (Figure 6). These effects of Berberine were found to be concentration dependent.

Berberine inhibits the NF- κB expression in SNU-1 cells

The effects of Berberine were also examined on the expression of NF- κB at 12.5, 25 and 50 μM by

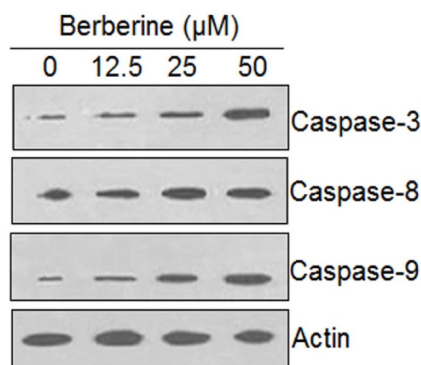


Figure 6. Berberine causes increase in the expression of Caspase-3, 8 and 9 at the indicated concentrations in SNU-1 cells as depicted by western blot analysis.

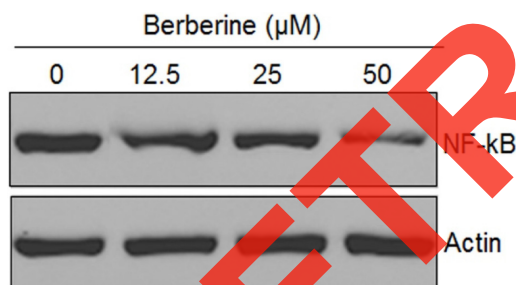


Figure 7. Effect of berberine on the expression of NF- κB in SNU-1 gastric cancer cells as depicted by western blot analysis. The experiments were performed in triplicate.

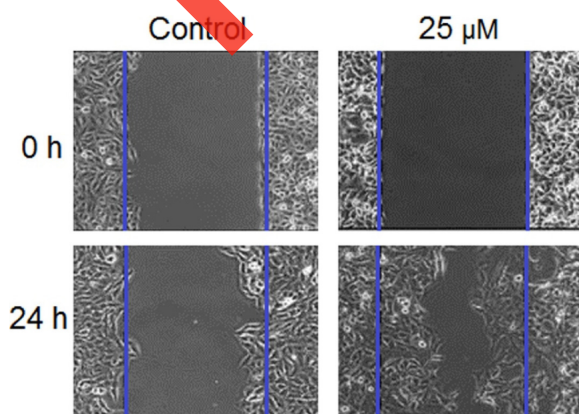


Figure 8. Wound healing assay showing the effect of berberine on the migration of the SNU-1 cells. The experiments were performed in triplicate.

western blot analysis. It was found that the expression of all NF- κB increased upon treatment of the SNU-1 gastric cancer cells with the molecule (Figure 7). These effects of Berberine on the expression of NF- κB were found to be concentration-dependent.

Berberine inhibits the cell migration of the SNU-1 cells

The impact of Berberine was examined on the migration and invasion of the SNU-1 cells by wound healing assay. The results showed that migration was considerably decreased upon the treatment of the SNU-1 cells with Berberine as evidenced from the wound width (Figure 8).

Discussion

Gastric cancer is devastating type of malignancy and around more than half of the cancers reported from East Asian countries are gastric cancers [8]. The incidence of Gastric cancer has significantly increased over the last few decades and varies geographically [9]. Plant-derived compounds have shown remarkable bioactivities which also include their potential to inhibit the growth of cancer cells [10]. Plant-derived molecules inhibit the growth of cancer cells via different molecular mechanisms such as triggering cell cycle arrest, apoptosis, necrosis and autophagy [11]. Moreover, many plant-derived molecules target specific signalling pathways to exert their anticancer effects. Studies carried out on plant-derived molecules have proved that molecules isolated from edible plants are comparatively safer and exhibit minimal or even no adverse effects [12]. Herein, the anticancer effects of a plant-derived alkaloid, Berberine, were examined against the human SNU-1 gastric cancer cells as well as the normal human GES-1 gastric cells. The results showed that Berberine dose-dependently inhibited the growth of cancer cells as exhibited an IC_{50} 25 μM . Nevertheless, it was interesting to see that Berberine exhibited minimal growth inhibitory effects on the normal human GES-1 cells and exhibited an IC_{50} of around 90 μM . These observations are in agreement with previous studies wherein Berberine has been reported to inhibit the growth of cancer cells, for example Berberine suppressed the proliferation of the epidermoid carcinoma cells via induction of apoptosis and cell cycle arrest [13]. In another study, Berberine inhibited the growth of hepatoma cells [14]. The JNK/p38 MAPK signalling pathway has been shown to be dysregulated in cancer cells and activation of this pathway has been reported to be involved in the development of different types of cancer cells [15]. Therefore, we also examined the

effects of this molecule on this pathway and interestingly it was found that Berberine could block this pathway concentration-dependently. Next, to further gain insights about the anticancer effects of Berberine on the SNU-1 cells, we performed DAPI and annexin V/PI staining and the results showed that Berberine caused apoptosis in the SNU-1 cancer cells which was also associated with activation of caspases and enhancement of the Bax/Bcl-2 ratio. A previous study has also shown that Berberine induces apoptosis in the U937 and B16 cells, further validating our observations [16]. Finally, the anti-metastatic effects of Berberine were examined by wound healing assay and was found that this molecule inhibited the migration of the SNU-1 gastric cancer cells which indicates the potential

of Berberine as an anticancer agent.

Conclusion

The results of the present study indicate that Berberine exerts significant anticancer effects on the human gastric cancer cells. The anticancer effects of Berberine are mainly due to apoptosis induction and blocking the JNK/p38 signalling pathway. Taken together, Berberine may be utilised in the development of systemic therapy for gastric cancer and deserves further studies.

Conflict of interests

The authors declare no conflict of interests.

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