Ind. J. Pharm. Edu. Res., 2025; 59(2):800-809.
https://www.ijper.org

Original Article

Pennogenin Induces Apoptosis in Colon Cancer HCT-116 Cells via Increasing Apoptotic Markers and Downregulating PI3K/AKT/mTOR Pathway

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ABSTRACT

Background: Colon cancer is the third major type of cancer globally and the second most reason for cancer-associated mortality. Natural products (phytochemicals) are considered safer alternatives to treat colon cancer. **Objectives:** In this work, we aimed at disclosing the anticancer activities of the pennogenin against the colon cancer cells. **Materials and Methods:** The antioxidant properties of pennogenin were investigated using numerous free radical scavenging tests, including DPPH and FRAP assays. The cytotoxicity of pennogenin (at concentrations ranging from 1-50 µM) against both colon cancer HCT-116 cells and non-malignant Vero cells was evaluated using the MTT test. The oxidative stress markers were determined using assay kits. The levels of ROS production, MMP level, and apoptotic levels were examined using specific fluorescence staining techniques. The levels of apoptotic proteins, cyclin D1, and PI3K/AKT/mTOR pathway protein levels were evaluated using corresponding assay kits. **Results:** The pennogenin efficiently reduced the free radicals, as determined by various techniques. The HCT-116 cell growth was significantly reduced after treating them with pennogenin, but the Vero cell growth was not affected by the pennogenin. The pennogenin treatment significantly enhanced the ROS accumulation, reduced the MMP, and induced cell death in the HCT-116 cells. The pennogenin treatment also elevated the levels of TBARS and reduced the antioxidant levels in the HCT-116 cells. Furthermore, the pennogenin also increased the apoptotic protein levels and reduced the cyclin D1 and PI3K/AKT/mTOR protein levels in the HCT-116 cells. **Conclusion:** The outcomes of this study highlighted that pennogenin successfully suppresses cell growth and enhances cell death in HCT-116 cells by increasing oxidative stress markers, apoptotic protein levels, and blocking of PI3K/AKT/mTOR pathway. As a result, it was clear that the pennogenin has the capacity to be an effective anticancer agent for effectively treating colon cancer.

Keywords: PI3K/AKT/mTOR pathway, Pennogenin, Apoptosis, Cyclin D1, HCT-116 cells.

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Received: 12-08-2024;
Revised: 06-11-2024;
Accepted: 24-02-2025.

INTRODUCTION

Colon cancer is the third major type of cancer globally and the second most reason for cancer-associated mortality. Most frequently, these malignancies originate from polyps in the colon that experience dysplasia and proceed to adenocarcinomas following the adenoma-carcinoma sequence. The cancer is characterized by the uninhibited proliferation of cells in the colon lining that ultimately result in the tumor development and, if left untreated, the spread of the cancer to other body parts.[1] Colon cancer is the second most prevalent tumor in women, responsible for nearly 13% of all cancer incidences, and the most common cancer in men, with a prevalence of around 17%. One of the key factors contributing to the global burden of colon cancer is the aging population. Additionally, other well-known causative factors for colon cancer include, smoking, over alcohol intake, less physical activity, increased use of red meat, obesity, and family history of the cancer.[2]

The pathogenesis of colon cancer is a multifaceted interplay of genetic, lifestyle, and environmental causes, all of which participate in the progression of this disease. Epidemiological data highlight that diet plays a key role in the etiology of colon cancer. A Western-style diet, defined by a increased utilization of red meat and processed meats, animal fats, and refined carbohydrates, coupled with a less intake of fiber, vegetables, and fruits, has been connected to an elevated risk of colon cancer.[3]

DOI: 10.5530/ijper.20250474

Furthermore, obesity, a hallmark of the Western lifestyle is also a major cause of colon cancer, with both body mass index and abdominal girth correlated with an increased risk. Environmental mutagenic factors, including carcinogen exposure, may also participate in the progression of colon cancer by inducing genetic changes in susceptible individuals.[4]

While conventional strategies including surgery, chemotherapy, and radiotherapy have made substantial progress in the treatment of colon cancer, drug resistance and toxicity remain significant problems.[5] Consequently, there is a growing interest in exploring alternative treatment approaches, including the use of natural products and phytochemicals. Natural products, like phytochemicals and dietary therapeutic candidates, are thought as safer alternatives to treat colon cancer. it has been highlighted that several natural products demonstrate potential anti-colon cancer activities and may be a alternative chemotherapy candidates.[6] Phytochemicals, which are biologically active compounds derived from plants, have shown promising results to prevent and treat colon cancer.[7] Given the challenges associated with conventional colon cancer treatments and the potential benefits of natural products, particularly phytochemicals, further investigation and development of these alternative approaches are warranted to enhance outcomes for patients with colon cancer. Pennogenin is a pivotal bioactive compound majorly found in the traditional medicinal plant *Paris polyphylla*.[8] It has been already reported that the pennogenin has several pharmacological effects such as anticancer,[9] antifungal,[10] and anti-obesity[11] activities. However, there are no scientific literatures to claim its anticancer effects against colon cancer. Hence, the current study was focused at disclosing the anticancer activities of the pennogenin against the colon cancer cells via down-regulating PI3K/AKT/mTOR pathway.

MATERIALS AND METHODS

Chemicals

The pennogenin, Fetal-Bovine Serum (FBS), DMEM medium, Dimethyl Sulfoxide (DMSO), antibiotics, and other chemicals were purchased from Sigma-Aldrich, USA. The assay kits for estimating the biochemical markers were purchased from Elabscience, USA and Abcam, USA, respectively.

Ferric-Reducing Antioxidant Power (FRAP) analysis

The FRAP analysis was executed as per the earlier published technique.[12] Pennogenin was added at diverse dosages (1-50 µM) along with 1 mL of FRAP solution, TPTZ reagent (10 mM), acetate buffer (300 mM), and ferric chloride (20 mM) reagent. The reaction solution (200 µL) was poured on a 96-well plate and sustained for 10 min. Subsequently, during the incubation, the resultant product in the reaction solution was examined using a wavelength of 593 nm.

DPPH activity

The impact of pennogenin on the removal of DPPH radicals was examined using the previously published procedure.[13] The DPPH reagent (150 µL) was prepared in ethanol with a amount of 0.25 mM. It was then mixed with diverse dosages of pennogenin at diverse concentrations 1 to 05 µM for 30 min at 37°C. Afterward, the absorbance was studied at 515 nm.

Chemiluminescence (CL) assay

The influence of pennogenin on the removal of superoxide radicals were assessed using the established methods.[14] The 10 µL of CL reagent was combined with different concentrations (ranging from 1 to 50 µM) of pennogenin, along with xanthine oxidase (80 µL) solution. The control solution was prepared using the HEPES buffer. Subsequently, the suspension was analyzed using a luminometer following the addition of 200 µL of hypoxanthine solution with a concentration of 0.72 mM. The scavenging effect was examined over 10 min, with measurements taken every 10 sec, using a luminometer.

Oxygen Radical Absorbance Capacity (ORAC) assay

An inhibitory effect of peroxyl radical-induced oxidation of pennogenin was assessed by measuring its ORAC capacity. This was done using a kit (Abcam, USA), following the guidelines of the manufacturer.

Cell culture maintenance

The HCT-116 cells was acquired from ATCC, USA, and cultivated on DMEM media supplemented with FBS (10%) and 1% antimycotic mixtures in a CO_2 (5%) chamber. Cells were collected when they achieved 80% confluency and used for subsequent fluorescence staining and biochemical assessments.

MTT cytotoxicity assay

To evaluate the influence of pennogenin on the growth of HCT-116 cells and Vero cells were evaluated using the MTT test. The cells were cultivated separately on a 96-well plate for 24 hr. Afterward, the cells were subjected to pennogenin treatment for an additional 24 hr at various concentrations of 1, 2, 5, 7.5, 10, 25, and 50 µM. Following the treatment, an MTT (20 µL) and DMEM (100 µL) was mixed for 4 hr. Ultimately, the absorbance was taken at 570 nm once the formazan depositions were dissolved using 100 µL of DMSO and analysed using an ELISA reader.

DCFH-DA staining

The accumulation of ROS in the cells treated with pennogenin was scrutinized by DCFH-DA staining. The cells were cultivated on a 6-wellplate and subsequently treated with pennogenin at doses of 7.5 and 10 µM for 24 hr. Subsequently, a DCFH-DA dye (10 µL) was mixed to the wells and sustained for 10 min. The

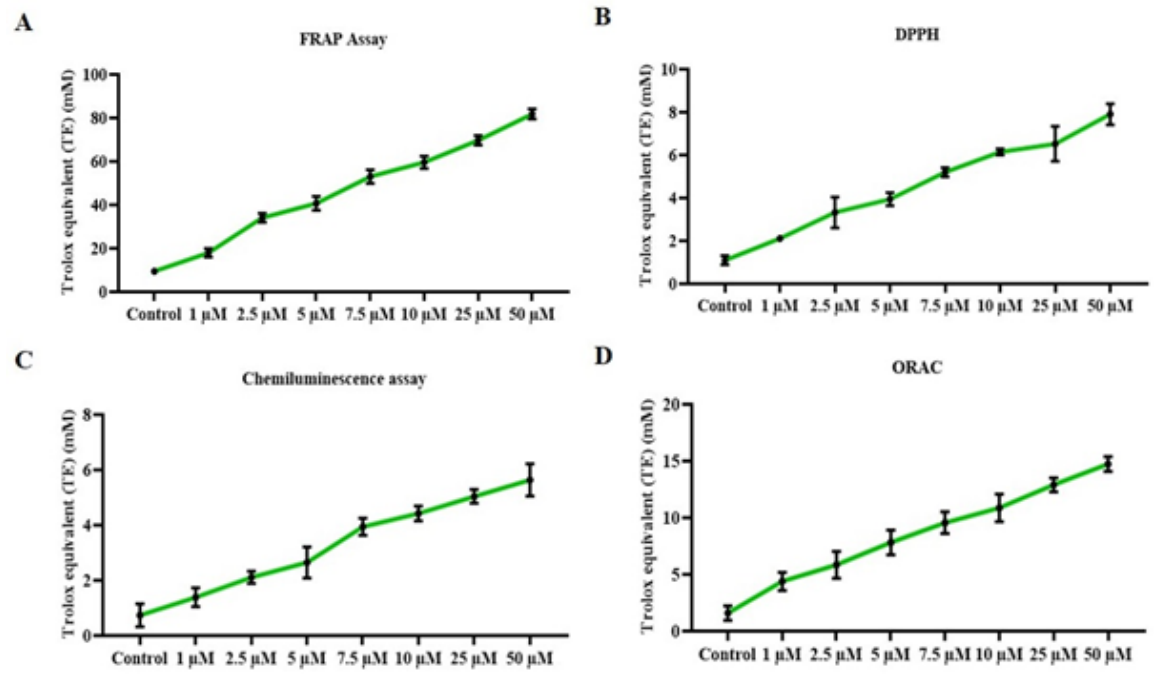

Figure 1: Effect of pennogenin on the *in vitro* scavenging of free radicals. The treatment with the different concentrations (1-50 µM) of pennogenin demonstrated its effective ability to scavenge free radicals. (A): FRAP assay; (B): DPPH assay; (C): Chemiluminescence assay; (D): ORAC assay. The results were demonstrated as the mean±SD of three independent assays. Using GraphPad Prism software, the one-way ANOVA and DMRT analysis were used to evaluate the final results.

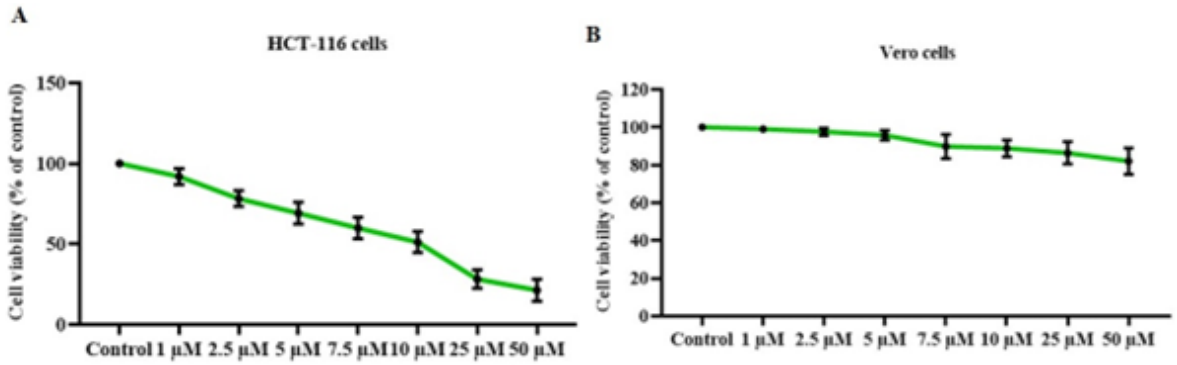

Figure 2: Effect of pennogenin on the viabilities of HCT-116 and Vero cells. The viability of the HCT-116 cells was significantly reduced and the Vero cell viability was affected by the by the various dosages of pennogenin (1-50 µM). The IC_{50} concentration of pennogenin was found to be 7.5 µM against HCT-116 cells. The results were demonstrated as the mean±SD of three independent assays. Using GraphPad Prism software, the one-way ANOVA and DMRT analysis were used to evaluate the final results.

fluorescence microscope was employed to evaluate the strength of the fluorescence, which is directly related to the ROS levels.

Rhodamine-123 staining

The MMP level in HCT-116 cells exposed to pennogenin were investigated using Rh-123 staining. In summary, cells were placed in a 24-well plate and treated with 7.5 and 10 µM of pennogenin for 24 hr. Later, the cells were stained using Rh-123 dye (10 µg/mL) for 30 min, then examined using fluorescence microscopy.

Propidium iodide staining

The apoptotic incidence in the pennogenin-treated cells was evaluated by PI staining method. The cells were placed on 6-wellplate and subsequently, cells were exposed to a 7.5 and 10 µM pennogenin for 24 hr. Next, a 5 µL solution of PI dye was applied and sustained for 20 min. The resulting fluorescence was then scrutinized using a fluorescent microscope.

Assay of oxidative stress markers

The HCT-116 cells from control and treated groups were collected, and their cell lysate was produced using cell lysis buffer to measure the oxidative stress marker levels. The TBARS, GSH, SOD, and CAT levels were measured in the cell lysates of pennogenin-exposed HCT-116 cells using the respective test kits, following the instructions given by the manufacturer of the kits (Elabscience, USA).

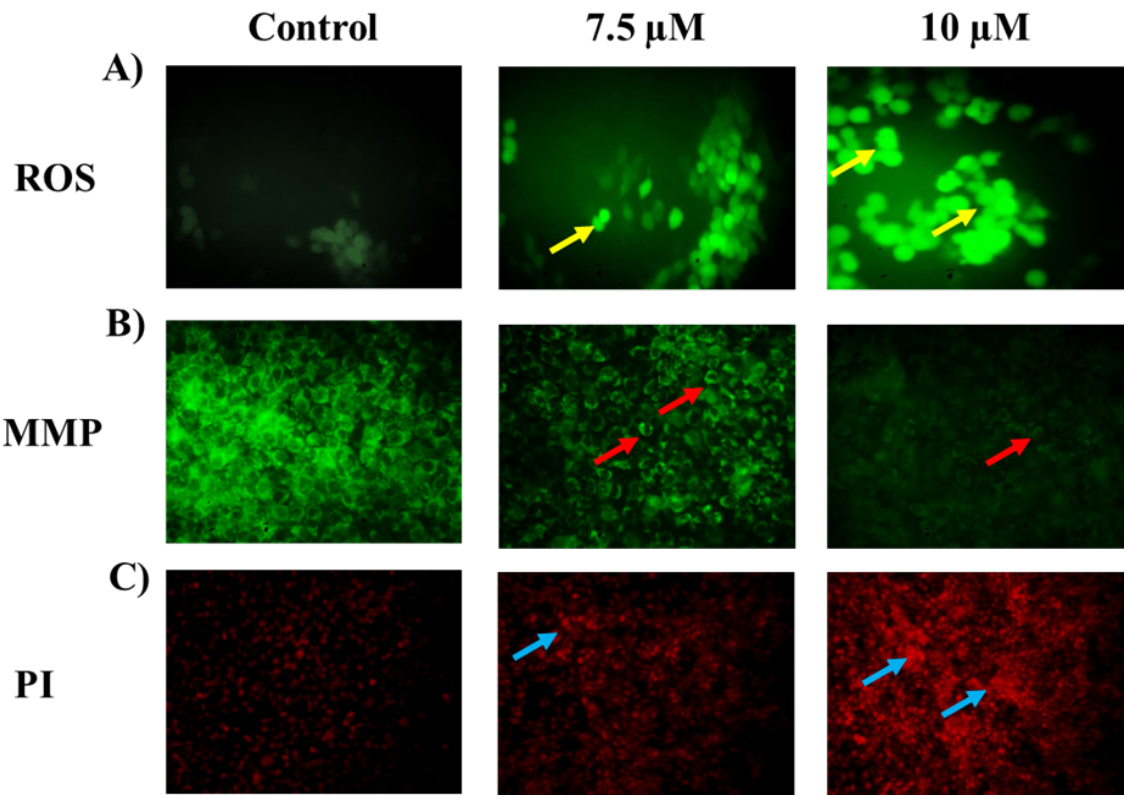

Figure 3: Effect of pennogenin on the ROS production, MMP level, and apoptosis in the HCT-116 cells. The green fluorescence of the 7.5 and 10 µM of pennogenin-treated HCT-116 cells were found to be higher than that of the control cells. This increased green fluorescence (yellow arrows) is positively correlated with increased endogenous ROS generation (A). The pennogenin treatment at 7.5 and 10 µM resulted in a reduction in green fluorescence (red fluorescence), indicating a significant drop in MMP levels. Higher green fluorescence (normal and intact MMP level) was seen in the control cells (B). The PI staining images also showed enhanced red fluorescence (blue arrows) than that of control cells, which indicates the presence of more apoptotic events, in the pennogenin-treated HCT-116 cells (C). The produced fluorescence intensity was measured using a fluorescent microscope.

Analysis of Bax/Bcl-2 and caspases levels

To examine the levels of apoptotic biomarkers, the cell lysate was obtained from the HCT-116 cells using a cell lysis buffer. The Bcl-2, Bax, caspase-3, and -9 levels were examined in the HCT-116 cells from both control and pennogenin-treated groups. The assays were performed using assay kits obtained from the manufacturer (Abcam, USA) and following the provided instructions.

Analysis of cyclin D1 and PI3K/AKT/mTOR protein levels

To study the levels of PI3K, AKT, mTOR, and cyclin D1 proteins, the cell lysate was prepared from the control and pennogenin-exposed HCT-116 cells. The PI3K, AKT, mTOR, and cyclin D1 protein levels were examined using commercially available test kits, and the guidelines provided by the manufacturer (Abcam, USA) were followed.

Statistical analysis

The statistical tests were conducted using Graphpad Prism software, and outcomes are given as the mean±SD of triplicate assays. The data was examined using one-way ANOVA and DMRT tests, with a significance level of $p<0.05$.

RESULTS

Effect of pennogenin on the *in vitro* scavenging of free radicals

The various tests were conducted to assess the free radical scavenging capabilities of pennogenin, and the findings are presented in Figure 1. Pennogenin exhibited a considerable reduction in free radical levels at increasing dosages. The pennogenin at 1 to 50 µM dosages effectively suppressed the generation of different types of free radicals. The *in vitro* activity of pennogenin to eliminate free radicals was evidenced by a reduction in these free radical levels when exposed to higher doses of pennogenin (Figure 1).

Effect of pennogenin on the HCT-116 and Vero cell viability

The influence of pennogenin on HCT-116 and Vero cells were examined by MTT test. The outcomes are presented in Figure 2. The treatment of pennogenin at different concentrations (1-50

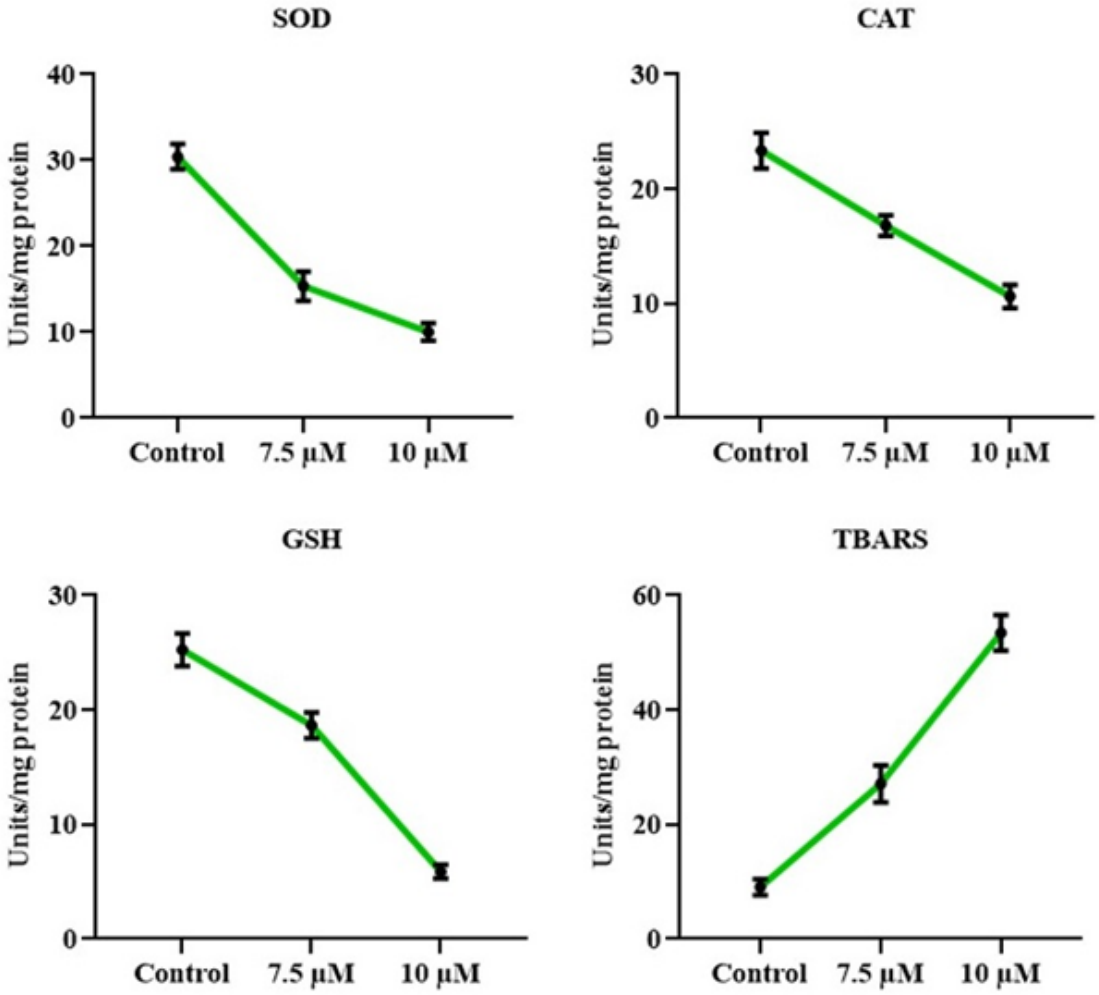

Figure 4: Effect of pennogenin on the oxidative stress marker levels in the HCT-116 cells. The results were demonstrated as the mean±SD of three independent assays. Using GraphPad Prism software, the one-way ANOVA and DMRT analysis were used to evaluate the final results.

µM) significantly inhibited the HCT-116 cell growth. Whereas, the growth of normal Vero cells was not significantly affected by the same amounts of pennogenin. These results present evidence of the cytotoxic properties of pennogenin on the proliferation of HCT-116 cells (Figure 2). The IC_{50} concentration of pennogenin for HCT-116 cells was found to be 7.5 µM. This concentration was selected for subsequent experiments.

Effect of pennogenin on the ROS generation, apoptosis, and MMP in the HCT-116 cells

Figure 3(A) illustrates the impact of pennogenin on the endogenous ROS production in the HCT-116 cells. The cells exposed to 7.5 and 10 µM of pennogenin illustrated a higher level of green fluorescence compared with control group. The enhanced green fluorescence indicates the enhanced endogenous accumulation of ROS in the pennogenin-exposed cells. Figure 3(B) displays the MMP status of pennogenin-treated HCT-116 cells. The control cells had a higher amount of MMP, as indicated by the strong green fluorescence. Nevertheless, the cells treated with a concentration of 7.5 and 10 µM of pennogenin exhibited a reduction in the level of MMP, as evidenced by the drop in green fluorescence (Figure 3B and 3C) depicts the apoptosis of both the control and pennogenin-exposed cells. An enhanced level of red fluorescence was seen in the cells treated with a concentration of 7.5 and 10 µM of pennogenin. The administration of pennogenin to HCT-116 cells demonstrated an elevated number of apoptotic cells, as evidenced by the stronger red fluorescence (Figure 3C).

Effect of pennogenin on the oxidative stress markers in the HCT-116 cells

The oxidative stress markers in the pennogenin-exposed HCT-116 cells were presented in Figure 4. The control cells exhibited increased GSH, CAT, and SOD levels, while a decrease in TBARS level was observed. Meanwhile, when cells were treated with 7.5 and 10 µM concentrations of pennogenin, there was a drastic elevation in TABRS level and a diminution in GSH, CAT, and SOD levels than the control group (Figure 4). According to these outcomes, it seems that pennogenin causes oxidative stress thereby facilitate the apoptosis.

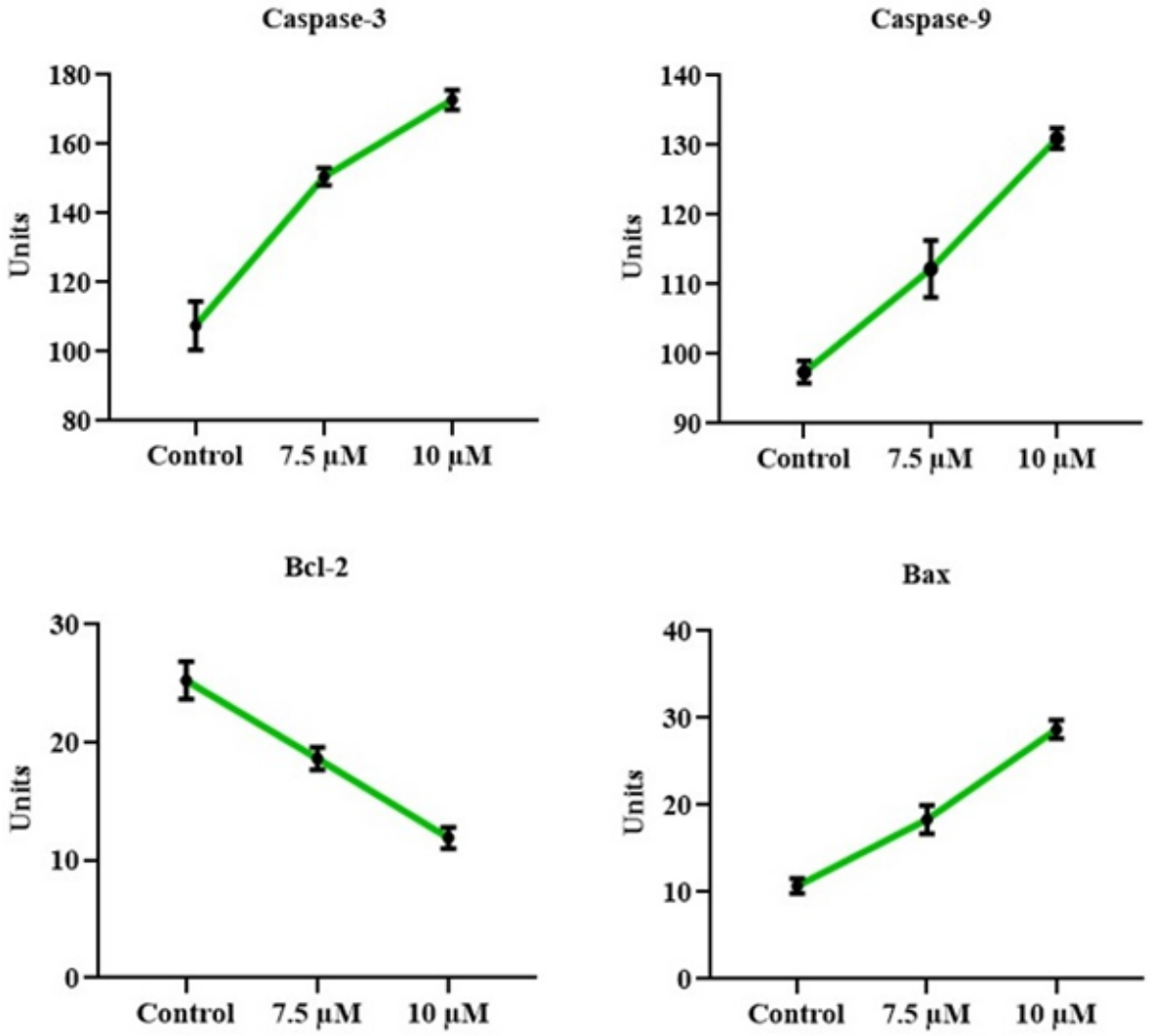

Figure 5: Effect of pennogenin on the apoptotic protein levels in the HCT-116 cells. The results were demonstrated as the mean±SD of three independent assays. Using GraphPad Prism software, the one-way ANOVA and DMRT analysis were used to evaluate the final results.

Effect of pennogenin on the apoptotic protein levels in the HCT-116 cells

Apoptotic protein expressions were analyzed in the pennogenin-exposed cells (Figure 5). In the control cells, the Bax, caspase-3, and -9 levels were decreased, however the expression of Bcl-2 was raised. Nevertheless, the treatment of 7.5 and 10 µM pennogenin to HCT-116 cells resulted in a substantial augmentation of Bax, caspase-3, and -9 activities, while the expression of Bcl-2 was diminished in comparison to the control group. Thus, it was clear that pennogenin treatment induces apoptosis in the colon cancer cells.

Effect of pennogenin on the cyclin D1 and PI3K/AKT/mTOR protein levels in the HCT-116 cells

Figure 6 displays the impacts of pennogenin on the cyclin D1 and PI3K/AKT/mTOR protein levels in the HCT-116 cells. The treatment of pennogenin at 7.5 and 10 µM concentrations significantly decreased the cyclin D1 and PI3K/AKT/mTOR proteins in the HCT-116 cells (Figure 6) when compared with control. Therefore, it was evident that pennogenin may block cell viability and initiate apoptosis in HCT-116 cells by blocking PI3K/AKT/mTOR protein levels.

DISCUSSION

Colon cancer, an aggressive type of cancer affects millions of people globally, is a complex and multifaceted condition that arises from a series of intricate cellular and genetic alterations. The progression from normal colorectal mucosa to invasive carcinoma is a well-documented process, characterized by distinct stages of transformation and the accumulation of various genetic and epigenetic abnormalities.[15] The pathophysiology of colon cancer is multifaceted, involving a manifold genetic and molecular changes that result in the transformation of normal colonic mucosa into invasive carcinoma.[16] Increase of genetic changes, such as mutations in key oncogenes is an essential step in the progression of colon cancer. These genetic alterations disrupt

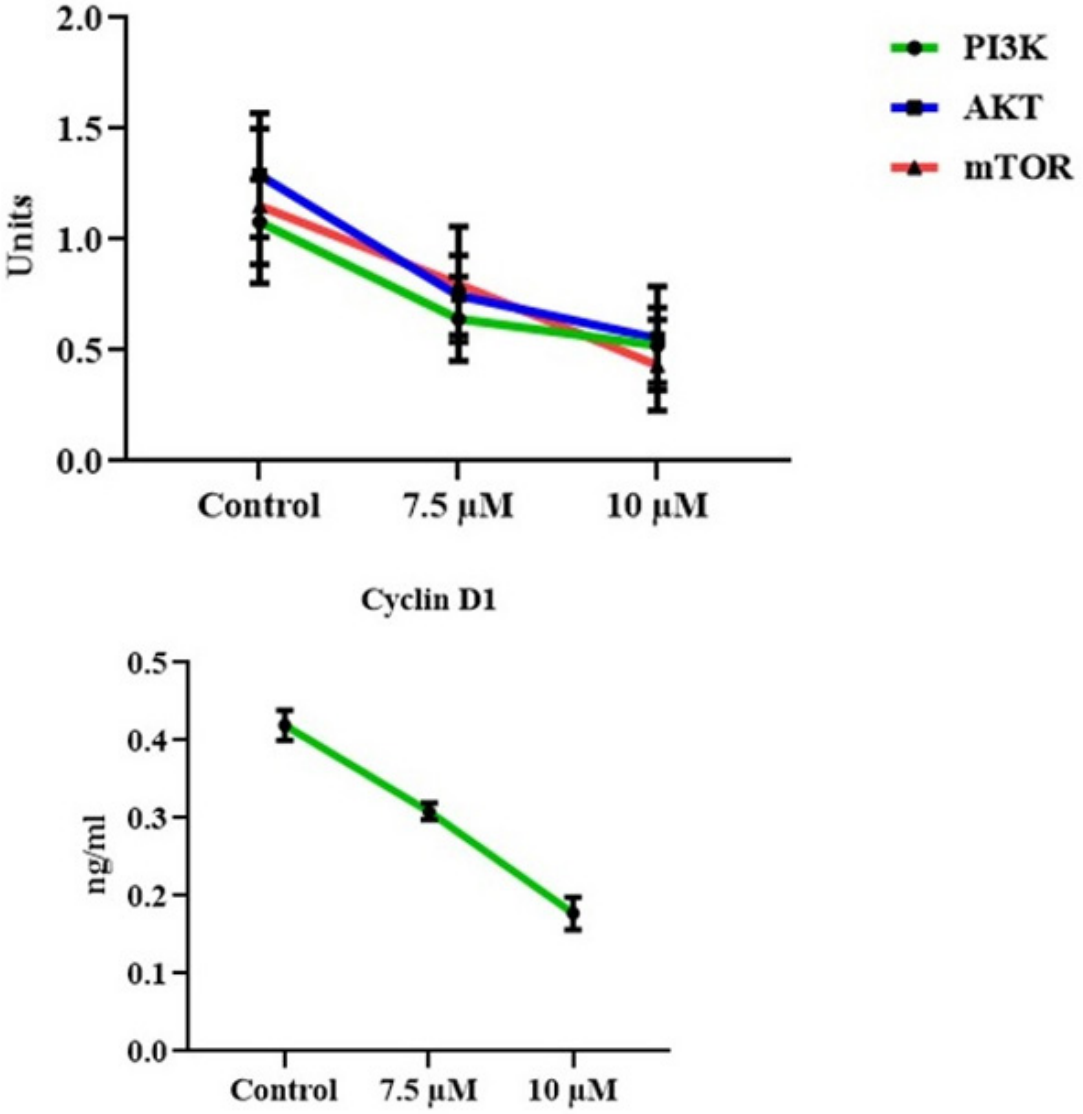

Figure 6: Effect of pennogenin on the cyclin D1 and PI3K/AKT/mTOR protein levels in the HCT-116 cells. The results were demonstrated as the mean±SD of three independent assays. Using GraphPad Prism software, the one-way ANOVA and DMRT analysis were used to evaluate the final results.

the equilibrium of growth and apoptosis, resulting in uninhibited cell growth and the progression of a malignant tumor.[17]

The development from normal colon mucosa to invasive carcinoma is a multifaceted mechanism, driven by the genetic alterations in key regulatory pathways. Dysregulation of specific biological mechanisms, including apoptosis, chromosomal instability, and disrupted metabolism, are critical causes in the transition from adenoma to carcinoma.[18] These genetic and epigenetic changes ultimately lead to the dysregulation of cellular mechanisms, including cellular proliferation, apoptosis, and angiogenesis, ultimately leading to the transition of non-malignant cells into malignant ones.[19] Colon cancer is a significant public health concern, with HCT-116 cells being a widely used *in vitro* model for studying the disease. Investigating the influence of bioactive compounds on the growth of these cells can provide valuable insights into potential therapeutic strategies. The current study focused to assess the impacts of pennogenin on the HCT-116 cell growth. The current outcomes proved that the pennogenin treatment successfully decreased the HCT-116 cells but did not affect the non-malignant Vero cells. The findings from this study can contribute to the ongoing efforts to develop more potential therapies for colon cancer.

ROS have emerged as critical players in the various cellular mechanisms that include cancer development and progression. Increasing proves highlight that the equilibrium between oxidative stress and antioxidant defense is an essential determinant in cancer cell survival and death.[20] Higher ROS accumulation can participate in cell proliferation, genetic instability, and metabolic adaptations that promote tumor growth. However, ROS can also trigger apoptosis, which can be exploited for cancer therapy. The induction of apoptosis is considered an effective strategy for cancer management, as apoptotic dysfunction is a phenomenon

of tumor cells.[21] Numerous studies have established that ROS can initiate both intrinsic and extrinsic apoptotic signaling in tumor cells. The extrinsic signaling is triggered by the activation of death receptors, whereas the intrinsic signaling is initiated by mitochondrial malfunction and the release of pro-apoptotic facotrs.[22,23] Compounds that modulate ROS levels may serve as potential chemotherapeutic agents or adjunctive treatments for cancer.[24-26] Similarly, the present findings proved that the pennogenin effectively elevated the endogenous ROS generation in the colon cancer cells.

The role of mitochondria in regulating apoptosis, particularly apoptosis, has been a subject of intense research in the area of cancer biology. Mitochondria are central players in the intrinsic apoptotic pathway, which is often dysregulated in cancer cells. The MMP is an essential factor in this process, as it influences the permeability of the mitochondrial membrane.[27] Cancer cells have developed various protective mechanisms to escape apoptosis, including the overexpression of anti-apoptotic genes or the inhibition of pro-apoptotic signaling pathways. Targeting these mitochondrial pathways has developed as a hopeful technique to treat cancer. The disruption of the MMP can lead to the cytochrome c release, initiating processes that culminate in apoptosis.[28] The MMP plays a key role in controlling of apoptosis, and its dysfunction is a hallmark of numerous pathological conditions, including tumor. Understanding the molecular mechanisms underlying the interplay between MMP and cancer cell apoptosis is expected to open new avenues for the development of targeted therapies.[29] In this work, the outcomes proved that the pennogenin substantially reduced the MMP levels in the HCT-116 cells, thereby facilitate the apoptosis.

The involvement of various oxidative stress biochemical markers, including TBARS and various antioxidants in the apoptosis of cancer cells has been extensively studied. TBARS is an oxidative stress marker, which can result in cellular damage and apoptosis. SOD, CAT, and GSH are important antioxidants that play a key role in protecting cells from oxidative stress.[30] Numerous studies have shown that the imbalance between these biochemical markers can participate in the progression of tumors. For instance, elevated TBARS and diminished SOD, CAT, and GSH levels have been associated with various types of cancer. Conversely, the upregulation of antioxidants has been linked to the stimulation of pro-survival signaling, which can result in the evasion of apoptosis and the progression of drug resistance.[31] Therefore, understanding the role of TBARS and various antioxidants in the apoptosis of tumor cells is essential to develop new therapeutic methods and the improvement of existing treatments. Moreover, the modulation of these biochemical markers has been studied as a therapeutic strategy to treat cancers. Several anti-cancer drugs have been developed that target the apoptotic pathways, with the goal of inducing or restoring apoptosis in cancer cells.[32] The present findings demonstrated that the pennogenin treatment significantly reduced the antioxidants and promoted the TBARS levels in the cells. Therefore, it was clear that the pennogenin may initiate apoptosis in HCT-116 cells via promoting the oxidative stress.

Apoptosis plays a key role in sustaining the equilibrium between cell growth and cell apoptosis, and its dysfunction is a hallmark of cancer development. The intrinsic signaling (mitochondrial pathway), is controlled by the Bcl-2 family of genes.[33] The pro-apoptotic genes, including Bax, enhance the cytochrome c release that stimulates caspase-9. Sequentially, caspase-9 triggers the executioner caspases, which further activates the proteolytic degradation of cellular components and the eventual disassembly of the cell.[34,35] The dysregulation of the apoptotic mechanism, including the anti-apoptotic gene overexpression's or the inactivation of pro-apoptotic genes, can result in the escape of apoptosis. Understanding the intricate balance between the pro- and anti-apoptotic genes is crucial for the advancement of therapies to restore the apoptotic machinery in cancer cells.[36] The outcomes of this work has proved that the pennogenin effectively promoted the Bax and caspases protein levels while reduced the Bcl-2 protein levels in the HCT-116 cells. Thus, it has been proven that the pennogenin can trigger apoptosis in colon cancer cells via regulating the pro- and anti-apoptotic protein levels.

The PI3K/AKT/mTOR signaling is a major controller of cell growth and metabolism, and its deregulation is participated in several cancer growth. Additionally, the cyclin D1 serves the pivotal role in cell cycle development and has been reported to inhibit apoptosis in tumor cells.[37] One of the signs of tumor is the capacity of tumor cells to evade apoptosis. Anoikis, a specific form of apoptosis initiated by the loss of cell-cell communications, is often subverted in tumor cells, permitting them to survive and proliferate in inappropriate microenvironments. Cyclin D1 has been found to promote anchorage-independent survival of tumor cells by blocking the anoikis-promoting function of FOXO transcription factors.[38] The PI3K/AKT/mTOR cascade is a key controller of cell growth and its activation is a common feature of numerous cancer types.[39] Moreover, the PI3K/AKT/mTOR pathway has been participated in the development of drug resistance in tumor, as it can enhance the survival of tumor cells in the face of antineoplastic therapies. Targeting this pathway has therefore become an attractive strategy for cancer therapy.[40] The interplay between the cyclin D1 protein and the PI3K/AKT/mTOR signaling plays an essential role in the evasion of apoptosis and the tumor progression. Comprehending the molecular processes of these markers is essential for the design of more effective cancer therapies. The outcomes of this study clearly proved that the pennogenin successfully down-regulated the PI3K/AKT/mTOR pathway and reduced the cyclin D1 levels in the HCT-116 cells. Hence, it has been proven that the pennogenin has the ability to block the PI3K/AKT/mTOR cascade thereby inhibit the colon cancer growth.

CONCLUSION

The findings of this study highlight that pennogenin successfully suppresses cell growth and enhances cell death in HCT-116 cells. Pennogenin treatment promoted the production of ROS, reduced MMP level, and promoted cell death by increasing the apoptotic protein levels while suppressing the Bcl-2. Furthermore, the pennogenin also reduced the cyclin D1 and PI3K/AKT/mTOR pathway in the HCT-116 cells. Thus, it has the capacity to be a potential anticancer agent to treat colon cancer. However, further research is necessary to understand the specific molecular mechanisms participated in pennogenin-induced cell death in HCT-116 cells.

ACKNOWLEDGEMENT

This work was supported by Traditional Chinese medical Hospital of Zhuji, Zhuji, Shaoxing, Zhejiang, 311800, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FBS: Fetal-bovine serum; **DMSO:** Dimethyl sulfoxide; **FRAP:** Ferric-reducing antioxidant power; **CL:** Chemiluminescence; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **ORAC:** Oxygen radical absorbance capacity.

SUMMARY

Colon cancer is the third major type of cancer globally and the second most cause of cancer-associated mortality. The present results highlighted that the pennogenin successfully down-regulated the PI3K/AKT/mTOR pathway and reduced the cyclin D1 levels in the HCT-116 cells. Hence, it has been proven that the pennogenin has the capacity to block the PI3K/AKT/mTOR signaling thereby inhibit the colon cancer growth.

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Cite this article: Zheng G, Gao D, Wang L, Zhou K. Pennogenin Induces Apoptosis in Colon Cancer HCT-116 Cells via Increasing Apoptotic Markers and Downregulating PI3K/AKT/mTOR Pathway. Indian J of Pharmaceutical Education and Research. 2025;59(2):800-9.