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Original article

Tilianin inhibits the human ovarian cancer (PA-1) cell proliferation via blocking cell cycle, inducing apoptosis and inhibiting JAK2/STAT3 signaling pathway

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ABSTRACT

Ovarian cancer is one of the deadliest gynecologic malignancies and is the seventh leading cause of mortalities and morbidities globally. Although there are various therapeutic strategies, a major challenge for scientific community is to come up with effective strategy to treat ovarian cancer. Tilianin, a polyphenol flavonoid is well known for its extensive biological actions like cardioprotective, neuroprotective, antioxidant, anti-inflammatory, anti-diabetic and anti-tumor properties. The current study is designed to investigate the anti-cancer action of Tilianin in ovarian cancer (PA-1) cells. The findings of this study revealed that Tilianin treatment results in significant and concentration dependent decrease in cell viability. The growth inhibiting action of Tilianin is associated with apoptosis which was confirmed by DAPI and AO/EtBr staining. The Tilianin-triggered apoptosis in PA-1 cells was correlated with elevated generation of ROS, loss of mitochondrial membrane potential, alterations in pro-apoptotic (upregulated mRNA expression of Bax) and anti-apoptotic (downregulated mRNA expression of Bcl2) factors and activation of caspase-8, −9 and −3. Cell cycle analysis revealed that Tilianin treatment prevented G1/S transition through reduced mRNA expression of cyclin D1. Additionally, the findings of this study also showed Tilianin inhibited JAK2/STAT3 signaling (downregulated expression of pJAK2, JAK2, pSTAT3, and STAT3) with no change in mRNA expression level of ERK indicating its non-involvement in the apoptotic and/or growth inhibition of ovarian cancer cells. In conclusion, the findings of this exploration provided clear evidence of anti-cancer effects of Tilianin in PA-1 cells through its anti-proliferative action, ability to induce apoptosis both through extrinsic and intrinsic pathways, cell cycle (G1/S) arrest and JAK2/STAT3 signaling inhibition.

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1. Introduction

Cancer multifactorial disease remains as a severe health issue affecting most peoples around the world (Le et al., 2018; Siegel et al., 2018). Cancer is a second most deadliest disease worldwide with increased morbidities and mortalities (Bray et al., 2018). Ovarian cancer (OC) has been considered to be one of most lethal gynecologic malignancies and is the 7th foremost reason of mortalities and morbidities globally (Sun et al., 2007; Smith et al., 2009; Momenimovahed et al., 2019). OC is extremely dreadful because of its asymptomatic nature that goes undetected during initial stages and hence 2/3 of patients are detected at III–IV stage of disease (Kurman and Shih, 2010). The risk of developing OC during the women's lifetime is 1 in 75 and her chance of death is 1 in 100 (Lewis et al., 2010). As per estimates there were 0.239 million cases and 0.152 million deaths globally and it was predicted that by 2035, the incidence rate and deaths will increase to 0.371 million and 0.254 million, respectively (World Ovarian Cancer Coalition Atlas, 2018). Surprisingly, the relative 5-year survival rate ranges between 30% and 40% worldwide (Allemani et al., 2015).

One of the best strategies to put an end to malignancy is to inhibit its proliferation (Abbaszadeh et al., 2019). The proliferation can be inhibited by arrest of cell cycle and/or initiation of apoptosis in cancerous cells (Wen et al., 2019). Cyclins and cyclin–dependent

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kinases (CDKs) take a key role to regulate cell cycle progression in mammalian cells. Activation of cyclin-CDK complex allows cell to move past particular check point and commits to unnecessary DNA replication and cell division. The G1/S transition has been considered to be most critical check point that is regulated by cyclin–CDK complexes. Among the G1 cyclins, cyclin D1 takes a prominent role in cell cycle regulation and its over-expression is connected to progression of ovarian cancer (Wang et al., 2019). On the other hand, induction of apoptosis is another possible way to curtail proliferation and/or progression of cancer cells in ovary (Mihanfar et al., 2019). Apoptosis or programmed cell death happens in two major pathways i.e., the intrinsic and extrinsic apoptotic pathways (Hassan et al., 2014). Furthermore, alterations in Bcl2 family members cause mitochondrial membrane destabilization, activation of caspase-3 and apoptotic cell death (Cheng et al., 2019). Additionally, accumulation of reactive oxygen species (ROS) is an another condition that is created in cells when they are undergoing stress and that leads to cell death, majorly by inducing apoptosis (Kao et al., 2017). Many previous evidences also highlighted the critical role of JAK2/STAT3 signaling in the initiation and differentiation of numerous cancers including ovarian cancer (Horvath, 2000; Siddiquee et al., 2007). Earlier it was reported that compounds that inhibit JAK/STAT signaling results in inhibited growth of ovarian cancer cells and apoptosis (Kim et al., 2019).

The current strategies to treat ovarian cancer include debulking surgery, radiation therapy, hormonal therapy, immunotherapy and chemotherapeutic drug treatment (Chandra et al., 2019). Platinum-based (carboplatin and cisplatin) and taxane-based (docetaxel and paclitaxel) drugs are generally prescribed chemotherapeutics to treat the ovarian cancer (Chandra et al., 2019). The major impediment to consider the usage of chemotherapeutics as choice of treatment is its harmful side-effects and thereby affects the quality life in cancer patients (Shapiro, 2016; Turcotte et al., 2017). Furthermore, the cancer patients may sometimes discontinue from the therapy (Shapiro, 2016; Turcotte et al., 2017). This has evoked the gates for intensive scientific research to find out novel cancer-therapeutics to manage ovarian cancer. Earlier, plethora of scientific evidences has demonstrated the potential of phytochemicals such as polyphenols, flavones and flavonoids to treat various types of cancers including ovarian cancer (Thomasset et al., 2007). Tilianin, a polyphenol flavonoid is known for its extensive biological actions like cardioprotective, neuroprotective, anti-oxidant, anti-inflammatory and anti-tumor effects (Akanda et al., 2018; Meng et al., 2018). Tilianin is reported to show anti-cancer effects through its cytotoxic nature and induction of apoptosis by down-regulating anti-apoptotic members, upregulating apoptotic members and stimulating caspases (Jiang et al., 2020). The present investigation was planned and performed to scrutinize the possible anticancer effects of tilianin against ovarian cancer PA-1 cells and its role and mechanisms of protection in terms of proliferation, cell cycle regulation and apoptosis through inhibition of JAK2/STAT3 signaling pathway.

2. Materials and methods

2.1. Chemicals and reagents

Human ovarian cancer cells (PA-1) were purchased from ATCC, Manassas, VA. Tilianin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,7-diacetyl dichlorofluorescein, Rhodamine 123, Acridine Orange, Ethidium Bromide were acquired from Sigma chemical Co., USA. While, the other chemicals of best analytical grade were bought from local commercial sources.

2.2. Cell culture

Human ovarian cancer PA-1 cells were used for this study and the same was procured from American Type Culture Collection (ATCC), USA. Cell propagation was done by adding Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (10%), penicillin (100 U/mL) and amphotericin B (2.5 μg/mL) and by maintaining in CO_2 incubator (37 °C temperature and 5% CO_2).

2.3. Cell viability assay

Cells from PA-1 cell line were loaded onto the culture plate at 1×10^4 cells/well population and kept for overnight incubation at 37 °C temperature and 5% CO_2. The cell lines were exposed to 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM/mL of Tilianin and kept in humidified condition for 24 h. After 24 h of incubation, MTT solution at a dose of 1 mg/mL was added and kept in incubator for 1–2 h and then stop solution (dimethyl sulphoxide) was added to each well and kept for 10 min in dark. Optical density readings measured at 570 nm were used to calculate the viability (percentage) of cells in Tilianin treated groups with respect to untreated control.

2.4. Measurement of ROS generation

The levels of ROS generation were evaluated by adding 2′, 7′-Dichlorofluorescein Diacetate (DCFH-DA) onto a cell culture plate containing 2×10^5 cells/well. The cells were supplemented without Tilianin, Tilianin at 15 μM/mL and Tilianin at 20 μM/mL and kept for incubation. After incubation, the fluorescence intensity was observed at 488 nm (excitation) and 530 nm (emission) wavelengths by using fluorescence microscope. The observed DCF intensity is relational to the level of ROS accumulation in the cell.

2.5. Assessment of mitochondrial membrane potential ($\Delta\Psi m$)

The $\Delta\Psi$m was determined by rhodamine 123 (Rh-123) staining method. Human ovarian cancer (PA-1) cells were cultured at 2×10^5 cells/well and administered with 30 and 40 μM/mL of Tilianin for 24 hr, underwent DAPI staining, treated with Rhodamine 123 (5 μg/mL), submitted to PBS washes and observed under fluorescence microscope.

2.6. Acridine Orange (AO) and Ethidium bromide (EtBr) staining

The percentage of survival and apoptotic cells were determined by using AO and EtBr staining method. 3×10^4/well were plated onto a 6-well plate and exposed to different dosages (30 and 40 μM/mL) of Tilianin for one day. Three parts of methanol and one part of glacial acetic acid were utilized to fix the cells for 30 min at 4 °C, submitted to PBS washes, underwent AO:EtBr (1:1) staining for 30 min at 37 °C and again cleansed with PBS. Floid cell imaging station used to count the survival and apoptotic cells.

2.7. DAPI staining of apoptotic nuclei

PA-1 cells were added onto a 6 well tissue culture plate and kept for overnight incubation for confluency. The cell lines were exposed to different dosages (30 and 40 μM/mL) of Tilianin for one day at 37 °C temperature and then fixed in paraformaldehyde (4%) for 30 min. The fixed cells were observed and captured under fluorescence microscope after staining with 0.5% DAPI for 15–20 min.

2.8. Caspase-9 and 8 activity assay

The caspase-8 and caspase-9 activities in PA-1 cells after they were exposed to Tilianin were performed by calorimetric assay kits following manufacturer's protocol (Mybiosource, USA). The PA-1 cells were cultured in humidified atmosphere for one day and then next 24 h the cells were exposed to Tilianin (30 and 40 μM/mL). After incubation, the cells were submitted to chilled PBS washes, treated with lysis buffer (50 μL) for 10 min, centrifuged at 10,000 × g for one minute and supernatant was utilized for the assay. The concentrations of protein in the samples were analyzed by Bradford method. After that, 50 μL of 2X reaction buffer (10 mM DTT and 4 mM DEVD-pNA substrate) was added to each sample and incubated for 2 h at 37 °C and then measured at 405 nm using an ELISA microplate reader.

2.9. RNA isolation and reverse Transcription-Quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from PA-1 cells using TRIzol reagent method as per instructions given by manufacturer (Thermo Fisher Scientific, Inc.). Next, cDNA was constructed using collected RNA samples by cDNA kits according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). The RT-PCR was carried out with the help of SYBR GREEN PCR master mix kit (Invitrogen, Karlsruhe, Germany) using Applied Biosystems Real-Time PCR System. The following sets of primers were used for RT-PCR. The samples were run in triplicates and for expression fold changes, Ct values were first normalized within the sample with housekeeping gene i.e., β-actin and then the relative mRNA expression was quantified by the 2-ΔΔCt technique.

2.10. Statistical analysis

Each sample was achieved in triplicates (n = 3) and conducted thrice to warrant the reproducibility of outcomes. The data were scrutinized by one way ANOVA after that Tukey's post-hoc assay and articulated as mean ± SD.

3. Results

3.1. Effect of Tilianin on cell viability in PA-1 cells

The cytotoxic effects of 0, 5, 10, 15, 20, 25, 30, 35, 0 and 45 μM/mL concentrations of Tilianin were depicted in Fig. 1. The results revealed that exposure of PA-1 cell line to different concentrations (5–45 μM/mL) of Tilianin resulted in significant dose dependent reduction in cell viability. Moreover, Tilianin at 30 and 40 μM/mL caused significant ($p < 0.01$) decrease in viability of cells with IC_{50} value of 20 μM/mL. Hence, doses 30 and 40 μM/mL were further used for the study.

3.2. Effect of Tilianin on ROS generation in PA-1 cells

The ROS generation was determined in ovarian cancer cells after treating them without or with Tilianin (30 and 40 μM/mL). Tilianin (30 and 40 μM/mL) treated PA-1 cells showed increased fluorescence intensity as an indication of increased generation of ROS levels when compared to untreated cells in a dose reliant manner (Fig. 2).

3.3. Effect of Tilianin on mitochondrial membrane potential (MMP) in PA-1 cells

Fig. 3 illustrates the effect of 30 and 40 μM/mL dose of Tilianin on MMP. The early indication of apoptosis i.e., loss of MMP was assessed by Rhodamine-123 dye. The untreated PA-1 cells (Fig. 3A) emitted an increased green fluorescence as an indication of no loss of membrane potential, while PA-1 cells were exposed to Tilianin at 15 (Fig. 3B) and 20 (Fig. 3C) μM/mL for 24 h showed reduction in green fluorescence indicating loss of membrane potential.

3.4. Effect of Tilianin on apoptotic morphological changes in PA-1 cells

Fig. 4 depicts the characteristic features of apoptosis in PA-1 cells after exposure to Tilianin. EtBr (red) penetrates into abridged nuclei of apoptotic cells, though healthy cells take AO (green) dye. The results showed increased green fluorescence in control cells is an indication of healthy live cells in untreated PA-1 cells. Conversely, increased red fluorescence was observed in 30 and 40 μM/mL concentrations of Tilianin administered PA-1 cells in a concentration reliant manner.

3.5. Effect of Tilianin on apoptosis in PA-1 cells

Fig. 5 shows apoptotic cells that are stained with DAPI. Supplementation of PA-1 cells with Tilianin (30 and 40 μM/mL) ensued in a significant increase in mean number of apoptotic cells in comparison with respective untreated PA-1 cells.

3.6. Effect of Tilianin on activities of Caspase-9 and Caspase-8

The caspase-9 and caspase-8 activities observed in control and Tilianin treated PA-1 cells are depicted in Fig. 6. Tilianin-treated PA-1 cells showed significantly elevated activities of caspase-9 and caspase-8 compared to that of untreated PA-1 cells.

3.7. Effect of Tilianin on mRNA expression of cyclin D1, Bcl2, Bax and Caspase-3

The gene expressions of cyclin D1, Bcl2, Bax and Caspase-3 are represented in Fig. 7. RT-PCR analyses revealed significantly downregulated expression of cyclin D1 (Fig. 7A) and Bcl2 (Fig. 7B) and upregulated expression of Bax (Fig. 7C) and Caspase-3 (Fig. 7D) genes in a concentration reliant manner after treating PA-1 cells with Tilianin (30 and 40 μM/mL).

3.8. Effect of Tilianin on mRNA expression of JAK2/STAT3/ERK

RT-PCR analysis showed that exposure of PA-1 cells to 30 and 40 μM/mL concentrations of Tilianin downregulated mRNA expressions of pJAK2, JAK2, pSTAT3, STAT3 and pERk with no change in expression level of ERK gene indicating induction of apoptosis in PA-1 cells through inhibition of JAK2/STAT3 pathway (Fig. 8).

4. Discussion

Ovarian cancer has been deliberated to be one of the noxious gynecologic malignancies worldwide. OC is extremely serious due to its asymptomatic nature during initial stages and 70% of the cases get diagnosed at late stages (stage III–IV) of disease (Kurman and Shih, 2010). Although current treatment strategies such as radiation therapy and/or chemotherapy are available to treat ovarian cancer, major concern is its undesirable side effects that affects the quality life of cancer patients (Shapiro, 2016;

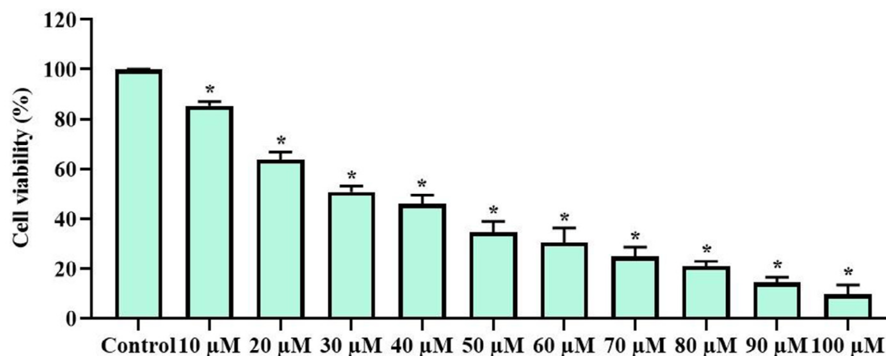

Fig. 1. Effect of different concentrations of Tilianin on cell viability of PA-1 cell line. Significant differences from control are indicated: $^*p < 0.05$.

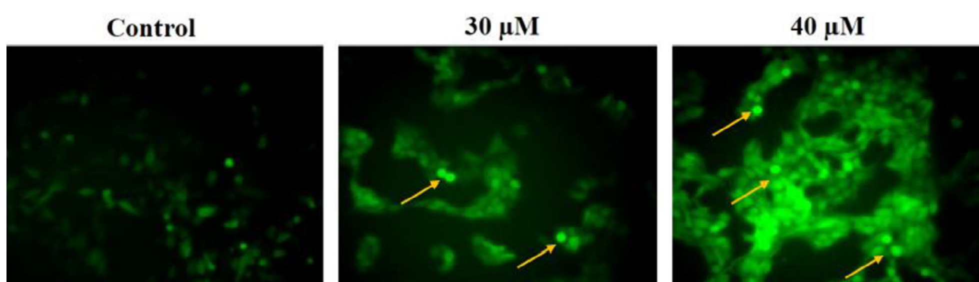

Fig. 2. Effect of Tilianin on intracellular ROS accumulation in PA-1 cells evaluated by DCFH-DA staining. Photomicrographs show the control, Tilianin 30 and 40 μM/mL administered PA-1 cells. Untreated PA-1 cells show weak DCF fluorescence. Tilianin (30 and 40 μM/mL) treated PA-1 cells show deep DCF fluorescence intensity as an indication of increased ROS generation (yellow arrows).

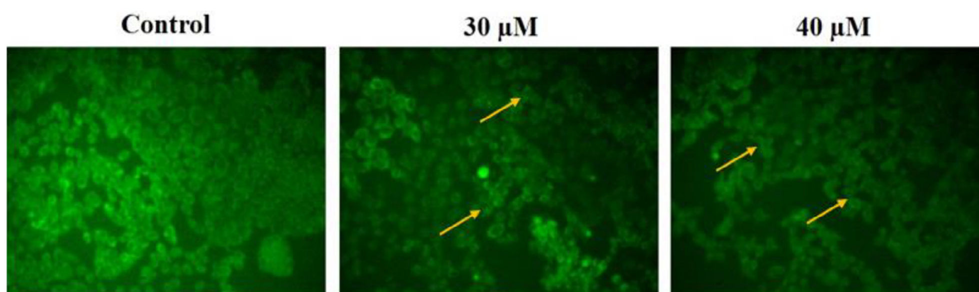

Fig. 3. Effect of Tilianin on mitochondria membrane potential ($\Delta\Psi m$) determined by rhodamine 123 (Rh-123) staining method. Photo micrographic images show the control (A) and Tilianin 30 (B) and 40 (C) μM/mL supplemented PA-1 cells. Untreated PA-1 cells show high fluorescence indicates polarized mitochondria membrane. Tilianin (30 and 40 μM/mL) treated PA-1 cells show decreased fluorescence indicate loss of membrane potential (yellow arrows).

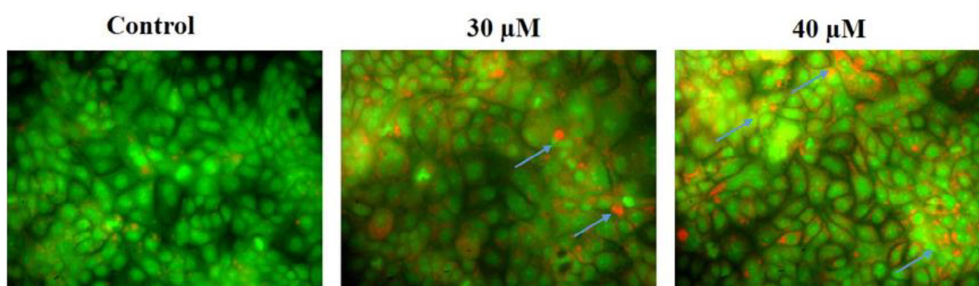

Fig. 4. Effect of Tilianin on apoptotic morphology stained by AO/EtBr staining method. Fluorescence microscopy images of control (A) and Tilianin 30 (B) and 40 (C) μM/mL administered PA-1 cells. Untreated PA-1 cells show increased survival cells and decreased apoptotic cells. Tilianin (30 and 40 μM/mL) treated PA-1 cells show decreased survival cells and increased apoptotic cells (blue arrows) in a dose reliant manner.

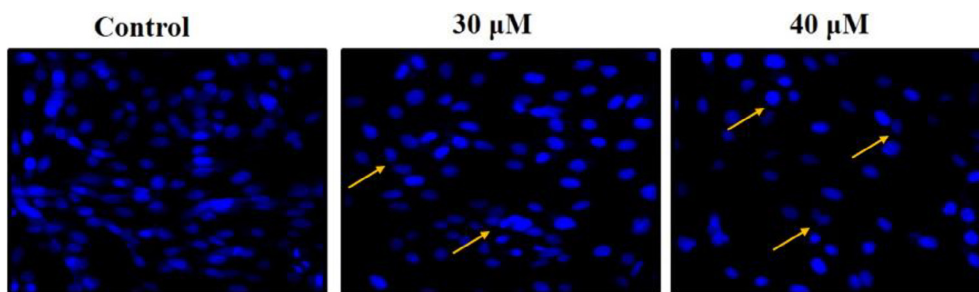

Fig. 5. Effect of Tilianin on apoptotic cells stained by DAPI method. Fluorescence microscopy images of control (A) and Tilianin 30(B) and 40 (C) μM/mL supplemented PA-1 cells. Untreated PA-1 cells show decreased apoptotic cells. Tilianin (30 and 40 μM/mL) administered PA-1 cells show augmented apoptotic cells (yellow arrows) in a dosage reliant manner. D) Percentage of apoptotic cells calculated.

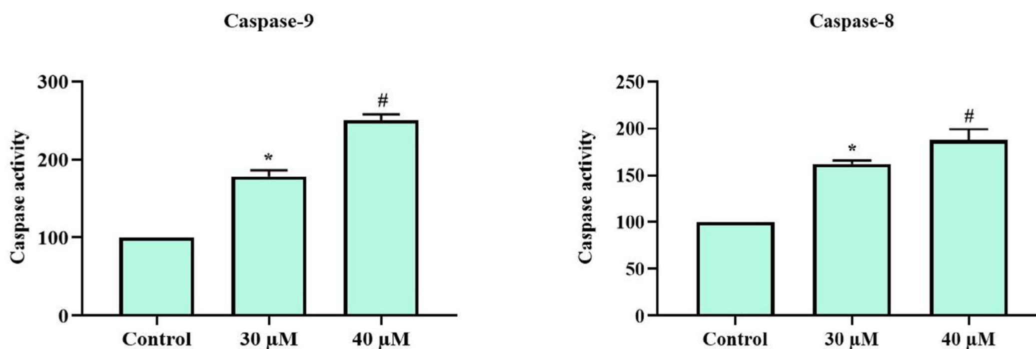

Fig. 6. Effect of Tilianin on caspase-9 (A) and caspase-8 (B) activities determined by ELISA method. Significant differences from control are specified: *$p < 0.05$, **$p < 0.01$.

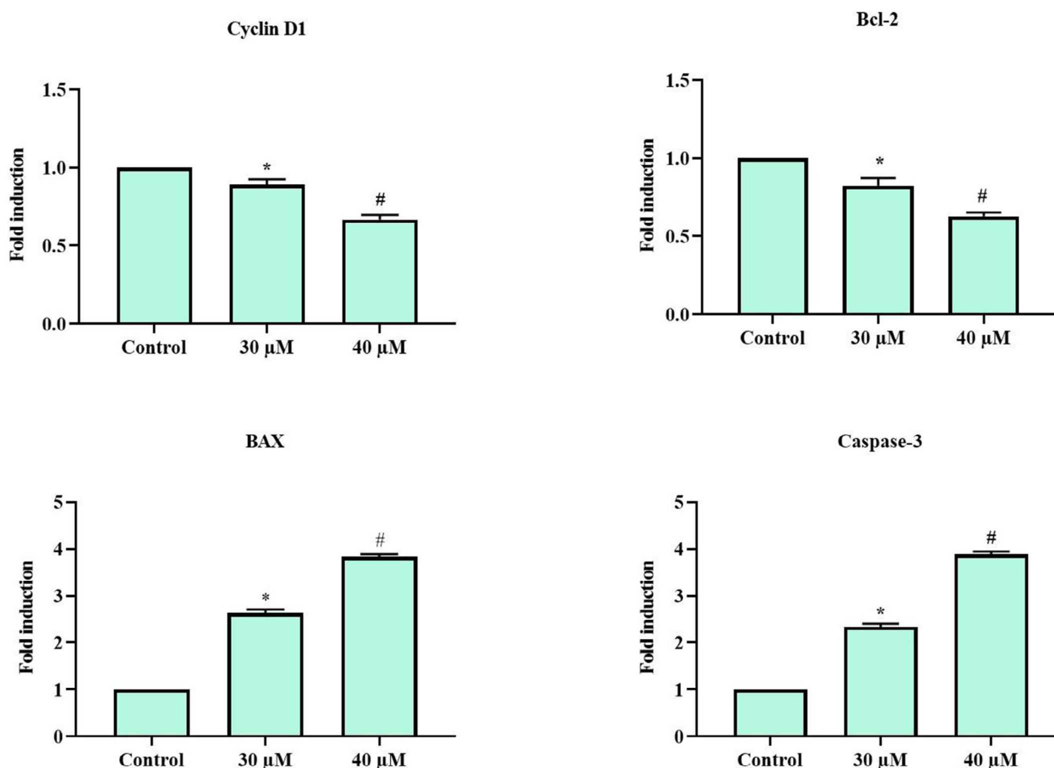

Fig. 7. Effect of Tilianin on mRNA expressions of Cyclin D1 (A), Bcl2 (B), Bax (C) and Caspase-3 (D) determined by RT-qPCR.

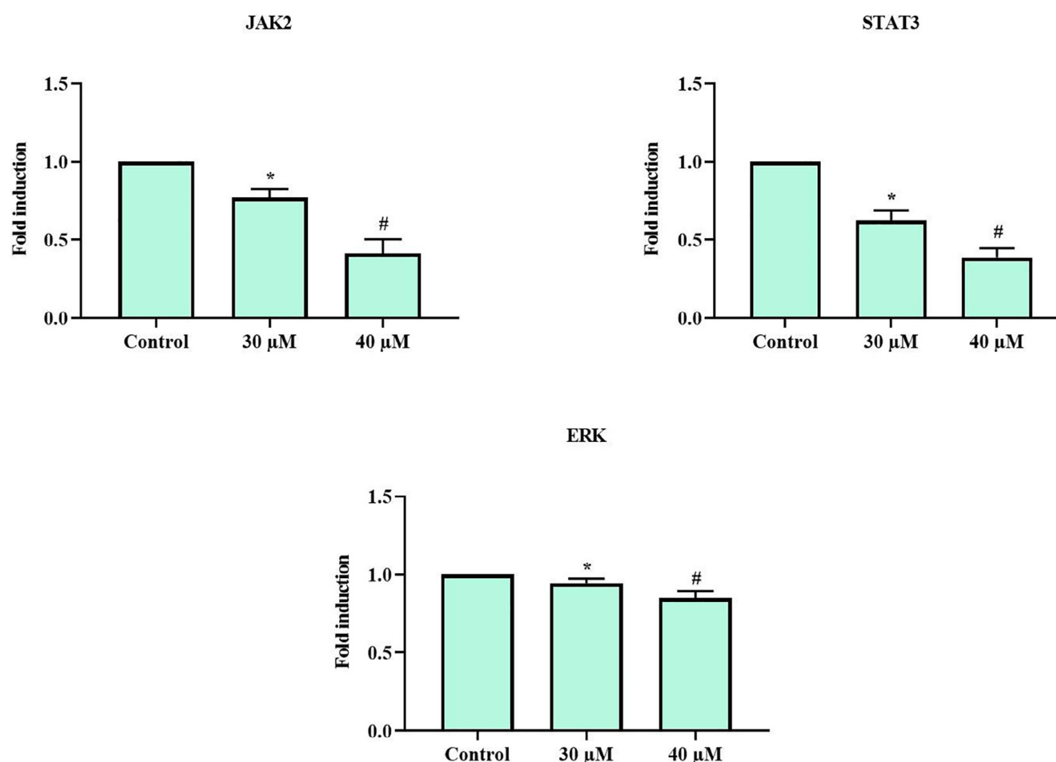

Fig. 8. Effect of Tilianin on mRNA expressions of JAK2, STAT3, STAT3, and ERK determined by RT-qPCR.

Turcotte et al., 2017). Hence, development of new treatment strategies is warranted. Plethora of scientific evidences pointed out the potential of phytochemicals to treat various types of cancers including ovarian cancer (Thomasset et al., 2007). During recent times, a polyphenol flavonoid i.e., Tilianin has attracted the scientific attention due to its wide variety of pharmacological properties and low toxicity (Akanda et al., 2019; Meng et al., 2018). The findings of this investigation validated for the first time that Tilianin supplementation shows anti-cancer assets in PA-1 cells via its capacity to lessen cell viability in a concentration reliant manner, induce ROS generation, damage mitochondrial membrane potential, induce apoptosis both through extrinsic and intrinsic pathways, arrest G1/S cell cycle progression and inhibit JAK2/STAT3 signaling.

The goal of anti-cancer research is to inhibit malignancy through inhibition of cancer cells proliferation (Abbaszadeh et al., 2019). In the present investigation, the cytotoxicity after treating PA-1 cells with Tilianin was assessed was assessed by MTT staining. Tilianin exerted significant cytotoxicity in PA-1 cells in a concentration (5–45 µM/mL) dependent manner. Furthermore, Tilianin at concentrations 30 and 40 µM/mL caused significant ($p < 0.01$) decline in viability of cells with IC_{50} value of 20 µM/mL. Considering this, doses 30 and 40 µM/mL were further used for the study. Supporting these observations, earlier it was reported that Tilianin treatment results in significant cytotoxicity in FaDu cells (Jiang et al., 2020) indicating the cytotoxic nature of Tilianin on cancer cells. Additionally, to know whether the cytotoxic effect of Tilianin is ascribed to its apoptotic effect, DAPI and AO/EtBr staining assays were performed. Interestingly, Tilianin treatment increased the DAPI and EtBr stained cells designating the apoptotic effect of Tilianin.

Apoptosis is an efficient way to eliminate potentially harmful cells through usage of cell intrinsic machinery. Apoptosis in mammalian cells generally happens in 2 pathways i.e., intrinsic and extrinsic pathways (Hassan et al., 2014). The caspase-8 and caspase-9 are activated in intrinsic and extrinsic apoptotic cascades, respectively (Mace et al., 2014; Kiraz et al., 2016; Ichim and Tait, 2016). In this study, expressively amplified activities of caspase-9 and caspase-8 were observed in Tilianin-treated PA-1 cells indicating the stimulation intrinsic and extrinsic apoptotic cascades. On the other hand, Bcl2 family proteins like pro-apoptotic (Bax) and anti-apoptotic (Bcl2) members are crucial for the mitochondria-mediated apoptotic pathway (García-Sáez (2020)). Alterations in Bcl2 family of proteins destabilize the mitochondria membrane potential, activate caspase-3 and leads to apoptotic cell death (Cheng et al., 2019). The results showed that Tilianin treatment results in significantly attenuated mRNA expression levels of pro-apoptotic (upregulated expression of Bax) and anti-apoptotic (downregulated expression of Bcl2) members. The findings of the present study also revealed upregulated expression of caspase-3 in Tilianin treated PA-1 cells. Supporting the findings of current study, previous findings also reported that treatment of cancer cells with Tilianin induced apoptosis through suppressed the antiapoptotic members (Bcl2), elevated pro-apoptotic members (Bax) and stimulated the caspase-3 activation (Jiang et al., 2020).

Mitochondria have long been considered to play a pivotal function in the energy metabolism of both normal and tumor cells (Chan, 2020). ROS are produced as part of normal cellular metabolism; low levels of ROS are crucial for normal redox signaling; excessive levels of ROS can destabilize the mitochondrial membrane potential (Dickinson and Chang, 2011) and cause cell death (Ahn et al., 2011). In this study, elevated generation of ROS levels, loss of MMP and augmented apoptotic cell death were observed after treating PA-1 cells with Tilianin. Earlier evidences also pointed out that the efficacy of many anticancer drug candidates is mainly because of excessive production of ROS levels, subsequent mitochondrial membrane damage and ensuing apoptosis in ovarian cancer cells (Liu et al., 2018; Vafadar et al., 2020).

Previously, it was also reported that proliferation of cancerous cells can also be inhibited by cell cycle arrest since proliferative cell transits past through cell cyle check points. In mammalian cells,

G1/S transition is considered to be the critical cell cycle check point and cyclin D1 takes the role to integrate extracellular proliferation signals to intracellular machinery. Overexpression of cyclin D1 is observed in various kinds of cancers including ovarian cancer (Wang et al., 2019). The overexpression of cyclin D1 reduces the spending time of cells in resting G phase and makes the cell past through S phase thereby leads to malignant proliferation (Jares et al., 1996). In this study, Tilianin treatment resulted in downregulated expression of cyclin D1 indicating its ability to halt cell cycle at G1 phase to inhibit proliferation.

A large number of scientific evidences highlighted the critical role of JAK2/STAT3 signaling in development and differentiation of various kinds of cancers including ovarian cancer (Horvath, 2000; Siddiquee et al., 2007). Previous studies also stated that inhibition of JAK2/STAT3 signaling by cancer therapeutics could lead to inhibition of ovarian cancer cell growth (Horvath, 2000; Siddiquee et al., 2007). Supporting earlier evidences, the results of the current study also proved that the antiproliferative effect of Tilianin on ovarian cancer cells is at least in part due to inhibition of JAK2/STAT3 signaling by downregulating pJAK2, JAK2, pSTAT3, and STAT3 gene expressions. The current findings also showed no alteration in mRNA expression level of ERK indicating its non-involvement in the apoptosis and/or growth inhibition of ovarian cancer cells by Tilianin.

Overall, the findings of the current study revealed that Tilianin treatment results in significant decline in viability of PA-1 cells in a dosage reliant mode. The anti-proliferative effect is correlated to apoptosis confirmed by DAPI and AO/EtBr staining. In addition, Tilianin induced apoptosis both through extrinsic and intrinsic (mitochondrial) pathways. The Tilianin-induced apoptosis is associated with elevated generation of ROS, loss of MMP, alterations in pro-apoptotic (upregulated mRNA expression of Bax) and anti-apoptotic (downregulated mRNA expression of Bcl2) members and activation of caspase-8, −9 and −3. Also, Tilianin arrested G1/S cell cycle progression in the form of reduced mRNA expression of cyclin D1. Additionally, Tilianin inhibited JAK2/STAT3 signaling with no involvement of ERK cascade. Altogether, the findings of the current investigation conveyed flawless suggestion of anti-cancer effects of Tilianin in PA-1 cells through its anti-proliferative action, ability to induce apoptosis both through extrinsic and intrinsic pathways, induction of ROS generation, loss of mitochondrial membrane potential, blocking of cell cycle (G1/S) progression, inhibition of JAK2/STAT3 signaling. The findings revealed that Tilianin could be utilized as a promising salutary agent for the management of ovarian cancer. However, in vivo studies are further warranted to better examine its potential role for ovarian cancer therapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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