RSC Advances

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Journal:	*RSC Advances*
Manuscript ID:	RA-ART-04-2015-006167.R1
Article Type:	Paper
Date Submitted by the Author:	28-May-2015
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The phytoestrogen genistein inhibits EGFR/PI3K/NF-kB activation and induces apoptosis in human endometrial hyperplasial cells

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Short title: Genistein inhibits EGFR/PI3K/NF-kB activation

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Abstract

Endometrial hyperplasia is an estrogen-dependent disease and is the most frequent precursor of endometrial cancer, diagnosed in pre- and peri-menopausal women. Aside from estrogenic induction, peculiar activation of the epidermal growth factor receptor (EGFR) signal is well known to coordinate with endometrial hyperplasia and its related carcinoma and could be an important factor in aetiology of endometrial hyperplasia. Genistein is an abundant isoflavone in soy, and play an important role in therapy of various diseases, however, the mechanism of action of genistein, towards endometrial hyperplasia is largely unknown. The current study was undertaken to explore the effect of genistein on cellular growth and EGFR -mediated signalling pathway in endometrial hyperplasia. Results demonstrated that genistein significantly suppressed the growth of human endometrial hyperplasial cells through EGFR inhibition and its downstream effectors PI3K/Akt and NF-κB. Genistein induced apoptosis in human endometrial hyperplasial cells through intrinsic pathway. Genistein also decreased NF-κB nuclear accumulation which regulates cellular proliferation and p53-dependent apoptosis. In conclusion, genistein inhibits cell proliferation through discontinued EGFR signalling, and induces apoptosis in primary endometrial hyperplasial cells via inhibiting the cell survival pathway PI3K/Akt and NF-κB.

Key words: genistein; EGFR; endometrial hyperplasia; apoptosis

1. Introduction

Genistein has been identified as the predominant isoflavone in soybean enriched foods[1]. In numerous epidemiological studies, this phytoestrogen has great potential as an inexpensive,

bioavailable chemotherapeutic agent with high antioxidant capacity as well as effective in the treatment of different types of cancer and diseases, like breast cancer[2], prostate cancer[3], ovarian cancer[4], colon cancer[5], endometrial cancer[6,7] and endometriosis[8]. Several investigations in various *in vitro* and *in vivo* models have demonstrated that genistein modulates estrogen sensitive parameters in selective estrogen receptor modulator (SERM) like manner and it may be referred as a phyto-SERM[9]. Thereby, genistein acts as a pleiotropic substance, which influences multiple actions that are involved in cell proliferation, cell invasion, anti-angiogenesis and apoptotic cell death via multiple intracellular signaling pathways[10-12]. Genistein inhibits EGFR- associated tyrosine kinase[13,14] and EGF-induced tyrosine phosphorylation, degradation of EGFR in HepG2 cells[15]. Genistein is capable of exhibiting NF-κB dependent- and NF-κB independent- apoptotic control via reactive oxygen species (ROS) generation depending on genetic cell types[12], and inhibits NF-κB activity through the MEK5/ERK5 pathway in breast cancer cells; it also suppresses cell proliferation and induces apoptosis[16].

Endometrial hyperplasia is a relatively common pelvic gynaecological condition that affects women of all age groups, with the majority of cases representing with abnormal uterine bleeding[17]. Endometrial hyperplasia is a condition of excessive and abnormal proliferation of the endometrial glands of the endometrium, or inner lining of the uterus through estrogen hormones[18]. It is a significant risk factor for the development or even co-existence of endometrial cancer with prolonged unopposed estrogenic stimulation and may also occur because of chronic disorder such as obesity, diabetes, polycystic ovarian syndrome and delayed childbearing[19]. The endometrial hyperplasia condition is commonly managed with progestin therapy with surveillance or hysterectomy[20,21]. Some women desire retention of fertility, in which case, standard surgical treatment, comprising hysterectomy, bilateral salpingo-oophorectomy and lymph node dissection is unacceptable, so careful monitoring

and treatment of women with this disorder is essential and there is a greater need for fertility-sparing treatments[22,23].

There are some reports on the inhibitory effect of genistein on endometrial hyperplasia, in randomized double-blind, placebo and progesterone-controlled clinical trials, and in premenopausal women[24-26]. However, the mechanism of action of genistein, towards endometrial hyperplasia is largely unknown. The current study was undertaken to explore the effect of the genistein on cellular growth and EGFR-mediated signalling pathway in endometrial hyperplasia. Our results demonstrated that genistein significantly suppresses the growth of human endometrial hyperplasial cells through inhibition of EGFR and its downstream effectors PI3K/Akt and NF-κB.

2. Materials and methods

2.1. Chemicals and antibodies

Genistein, [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] (MTT) , collagenase, DNase, propidium iodide (PI), Annexin V-FITC (fluorescein isothiocyanate)-labeled apoptosis detection kit were purchased from Sigma-Aldrich, USA. All culture media and other reagents were also purchased from Sigma-Aldrich, USA. Anti-cytokeratin-7, -ERα, -ERβ, -PR, -PCNA, -c-Fos, -c-Jun, -β-catenin, -IGF-1, -p-Bad (ser 112), -Bad, -Bim, -PUMAα, -NOXA, -p-CREB (ser 133), -CREB, -p53, -p-Mdm2 (ser 166), -Mdm2, -pPI3K (tyr 485), -PI3K, p-Akt (ser 473), -Akt, p-NF-κB p65 (ser 536), -NF-κB p65, -β-actin antibodies, peroxidase- and fluorescein isothiocyanate (FITC)-conjugated secondary antibodies were procured from Santa Cruz (Dallas, TX, USA). Antibodies for cleaved caspase-3 and -9, cleaved PARP, Bax, EGFR, p-EGFR (tyr 1173), Bcl-2, and $Bcl_{XL,}$ were

purchased from Cell Signalling Technology, Life Sciences (Boston, MA, USA). Fig.1 shows the chemical structure of genistein.

2.2. Endometrial tissue collection and primary culture of endometrial cells

Endometrial hyperplasia samples were collected in the operating room of the Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, Uttar Pradesh, India. Endometrial hyperplasia samples (five different cases of atypical hyperplasia) were collected from patients with abnormal uterine bleeding (age: 25–40 years). Normal endometrial samples (three different cases) were collected from the patients undergoing hysterectomy for uterine prolapse reasons. A specific informed consent was obtained from each patient and the study was approved by the local Human Ethics Committee. Histopathological testing were carried out by expert gynecologists and pathologists from Department of Obstetrics and Gynecology and Department of Pathology, King George's Medical University, such as gland-to-stroma ratio, gland's shape and size, nuclear/cytoplasmic ratio, cytologic atypia, hyperchromatosis by simple staining and expression of estrogen receptor, progesterone receptor and proliferation marker (Ki67) by immunohistochemistry, who approved the category of samples as hyperplasia to be of atypical type and after the testing and evaluation, tissue samples were taken for further studies.

Briefly, tissue were collected in MEM, minced in 1 mm pieces and incubated with 1 mg/ml collagenase and DNase (2 mg/ml) in MEM for 2 h at 37ºC with regular mixing. Digested tissue was mechanically dissociated through a 1 ml tip and resuspended in 2 ml of fresh MEM. The cells were separated from tissue clumps and debris by filtration through an 18-mesh sterile gauze and centrifugation, washed twice with MEM containing 10% fetal bovine

serum, 1 mM sodium pyruvate, 2 mM L-glutamine, and 2% of antibiotic-antimycotic solution (Sigma–Aldrich, USA) and then transferred into tissue culture flasks (75 cm^2, Corning, USA). Cells were incubated at 37ºC with saturating humidity and 5% CO_2. Prior to experiments, cells were cultured in phenol red-free MEM supplemented with 10% charcoal stripped fetal bovine serum and 1% antibiotic-antimycotic solution[27,28]. The human endometrial hyperplasial cells were characterized by examining the expression of cytokeratin-7 (an epithelial marker) by immunocytochemistry. (Supplementary Fig.1)

2.3. Cell viability assay

Cell viability was determined by MTT assay. Cells were seeded (3×10^3 cells/well) into 96-well plate and treated with genistein (25, 50, 100, 150, and 200 μM) for 48 h. At the end of incubation, MTT (0.5 mg/ml) (Sigma) was added and incubated for 2 h at 37ºC. After 2 h of incubation, supernatants were removed and 100 μl of DMSO was added. The formazan crystals formed inside the viable cells were solubilized in DMSO and the OD was read with Microquant (Biotech, USA) at 540 nm. The IC_{50} values for each compound were determined by Compusyn software. The experiments were performed three times with five replicates in each.

2.4. ELISA for EGFR activation

Levels of phosphorylated and total EGFR were quantified by using ELISA kit (Invitrogen). In brief, primary endometrial hyperplasial cells were treated as indicated in Fig. 3 B. At the end of incubation, cell lysate was prepared by lysing the cells in buffer containing 10 mM Tris pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM

Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS and 0.5% deoxycholate supplemented with protease and phosphatase inhibitors. EGFR activation was measured by following the manufacturer's instructions. OD was taken with Microquant ELISA reader (Biotech, USA) at 450 nm. The experiments were repeated three times with three replicates in each.

2.5. Co-immunoprecipitation assay

Interaction between EGF ligand and EGRF proteins was studied by co-immunoprecipitation of the complex followed by immunoblotting. Briefly, 2 µg anti-EGFR antibody was added to 500 µg of cell lysate and samples were incubated for overnight at 4ºC. In negative control, cell lysate was incubated with related non-immune serum instead of anti EGFR. Then 100 µl of Protein A-Sepharose beads (Sigma-Aldrich, USA) suspension was added and samples were incubated for 1 h at 4ºC with constant rocking. Immunoprecipitated complexes were collected by centrifugation at 3000×g for 2 min at 4ºC and then washed three times with RIPA buffer (Sigma-Aldrich, USA), then resuspended in Laemmli sample buffer to a final concentration and heated for 5 min at 95ºC. The supernatants were collected by centrifugation at 12,000×g for 30 s at room temperature. Equal amounts of immunoprecipitated proteins were separated by 8% SDS-PAGE and transferred on PVDF membrane (Millipore). The proteins were probed with anti-EGF, followed by the related secondary peroxidase conjugated antibody. Antibody binding was detected by using enhanced chemiluminescence detection system (GE Healthcare). Bands were detected by Gel Doc imaging system (Bio-Rad) and analyzed by densitometry using Quantity One Software (v. 4.5.1).

2.6. Annexin-V/propidium iodide labeling and flow cytometry assay for apoptosis

Cells (2×10^5 cells/ml) were cultured in 6-well plates and treated with genistein (50 μM and 100 μM,) for 48 h. Adherent and non-adherent cells were probed with FITC-conjugated Annexin-V and PI for 15 min. The staining profiles were determined with FACScan and Cell-Quest software. The experiments were performed three times.

2.7. Measurement of Mitochondrial Membrane Potential (MMP)

In brief, human endometrial hyperplasial cells were treated with genistein (50 and 100μM) for 48 h and harvested by trypsinization. Cells were incubated with 2 mL of medium containing JC-1 dye (1 μg/mL) for 15 min at 37 °C. Stained human endometrial hyperplasial cells were washed with PBS and subjected to flow cytometry analyses as per standard protocol using FL1, and FL2 channel. The experiments were performed three times.

2.8. Immunofluorescence imaging by florescence microscopy and confocal microscopy

Cells were grown on coverslips in 12-well plate and treated with vehicle, 50 and 100 μM of genistein in primary endometrial hyperplasial cells and normal endometrial cells for 24h. Cells were then fixed in methanol and acetone in 1:1 ratio at 4ºC and permeabilized with 0.1% Triton X-100. Cells were washed with PBS and blocked with 1% BSA and incubated with p-EGFR, EGFR, and NF-κB antibody for overnight followed by 1h incubation with fluorescence-tagged secondary anti-rabbit antibody, then counterstained with DAPI for 5 min. Images were captured at 40X with the NIS-Elements F 3.0 camera and 63X using Carl Zeiss LSM 510 META microscope and analysed using LSM-Image-Examiner Software to

detect fluorescence and DAPI emissions. In negative controls, cells not exposed to primary antibodies.

2.9. Western blot analysis

Endometrial primary culture cells were treated with vehicle or 50 and 100 μM concentrations of genistein for 48 h. After each treatment, cells were lysed in lysis buffer (Sigma-Aldrich, USA) supplemented with a protease inhibitor cocktail (PIC). Equal amounts of protein were separated by gel electrophoresis and transferred to Immuno-Blot PVDF membrane (Millipore). The membrane was blocked for 1 hr in 5% skimmed milk and incubated with primary antibody overnight at 4ºC. The membranes were then incubated with secondary antibody for 1 h. Antibody binding was detected by using enhanced chemiluminescence detection system (GE Healthcare). After developing, the membrane was stripped and re-probed with β-actin, Bad, CREB, Mdm2, PI3K, Akt, and NF-κB p65 antibodies. Quantitation of band intensity was performed by densitometry using Quantity-One software (v.4.5.1). Each experiment was performed three times.

2.10. Real-time polymerase chain reaction

Total RNA from genistein treated and untreated endometrial hyperplasial cells was extracted using the Tri-reagent by following the manufacturer's instructions. cDNA was synthesized from 5 μg of total RNA using first strand cDNA synthesis kit. The quantification of the selected genes by real time-PCR was performed with a LightCycler (Roche). The nucleotide sequences of the primers used have been given in Supplementary Table 1. Expressions of the investigated genes were compared to the steady expression of GAPDH. The PCR system was

programmed according to the manufacturer's instructions. The experiments were repeated three times.

2.11. Statistical analysis

Results are expressed as Mean ± S.E. for at least three separate determinations for each experiment. Statistical significance was determined by ANOVA and Newmann Keul's test. P values less than 0.05 were considered significant.

3. Results

3.1. Genistein inhibits endometrial hyperplasia cell viability

The effect of genistein on cell viability was examined by MTT assay. Genistein reduced the viability of human endometrial hyperplasial cells in a dose-dependent manner with IC_{50} of ~75 μM ($P< 0.001$), This showed that genistein has an anti-proliferative effect on primary endometrial hyperplasia cells without affecting the normal endometrial cells (Figure 2 A, B and D).

3.2. Effect of genistein on expression of proliferation markers

For analysis of proliferation marker proteins and mRNA expression, human endometrial hyperplasial cells and normal endometrial cells were treated with 50 and 100 μM genistein. A significant reduction in ERα, PR, PCNA, β-catenin, IGF-1, c-fos, and c-jun protein was

observed in dose-dependent manner, whereas significant induction was observed in the expression of ERβ in human endometrial hyperplasial cells. The densitometric analysis showed that in human endometrial hyperplasial cells, genistein caused reduction by ~ 50 % in ERα ($p < 0.001$) , ~ 52 % in PR ($p < 0.01$), ~ 48 % in PCNA ($p < 0.001$), ~ 50 % in β-catenin ($p < 0.001$), IGF-1 ($p < 0.001$), c-fos ($p < 0.001$), c-jun ($p < 0 .001$), whereas ERβ expression was induced by ~ 45% ($p < 0.001$) at 100 μM concentration (Fig.2 C). Also, the dose- dependent effects were observed on expression of proliferative markers (Supplementary Fig. 2). No significant changes was found in the levels of ERα, PR, PCNA, IGF-1 protein expression in human normal endometrial cells treated with genistein (Supplementary Fig.3).

Besides this, we also studied the effect of genistein on mRNA expression of proliferative markers and found a reduction of ~ 0.7 fold in ERα, ~ 0.6 fold in PR and PCNA, ~ 0.5 fold in β-catenin expression at 100 μM concentration (Fig.2 E). These results show that the reduction in protein levels under the influence of genistein, was due to the inhibition at their respective mRNA expression levels and not due to protein degradation.

3.3. Genistein inhibits EGFR activation, EGF-EGFR interaction and antagonises EGF-induced EGFR activation

To identify the molecular mechanism responsible for genistein toxicity, we sought to investigate the diverse biological responses triggered in primary endometrial hyperplasial cells by the genistein. Since the genistein was found to inhibit proliferation of endometrial hyperplasial cells, we went onto see if the genistein also prevents binding of EGF to EGFR . Co-immunoprecipitation studies indicated that genistein like EGFR inhibitor significantly

reduced the formation of EGF-EGFR complex ($p<0.01$) and in presence of EGF ($p<0.001$) (Fig. 3 A).

Furthermore, we analyzed the effect of genistein on p-EGFR (tyr 1173) and total EGFR activation using ELISA, and immunocytochemistry (20X) (Fig. 3 B, and C). At 50 and 100 μM concentration, the genistein significantly inhibited phosphorylation of EGFR and more significant at 100 μM ($p < 0.001$). No significant change in the level of total EGFR was observed in the human endometrial hyperplasial cells on treatment with indicated concentrations of the genistein.

3.4. Genistein down regulates the expression of NF-κB p65 and its nuclear localization in endometrial hyperplasial cells

Genistein was found to reduce the nuclear localization of NF-κB in endometrial hyperplasial cells without affecting normal endometrial cells (Fig. 6 A and B). It also reduced the expression of NF-κB in endometrial hyperplasial cells (Fig. 6 A). Phosphorylation status of NF-κB (ser 536) was significantly suppressed by genistein in endometrial hyperplasial cells. The densitometric analysis showed that genistein reduced the expression of p-NF-κB by 50% ($p <0.001$), at a concentration of 100 μM of genistein.

3.5. Genistein induces p53-dependent apoptosis in human endometrial hyperplasial cells via intrinsic pathway

In order to check if the loss in cell viability on treatment with the genistein is due to induction of apoptosis, we analyzed Annexin V/PI stained cells by flow cytometry. Genistein increased the percentage of apoptotic cells at 100 μM and apoptotic cell fraction was approximately ~

35 % higher in comparison to control (Fig. 4 A). We also checked protein expression of pro-apoptotic markers Bax, Bad, Bim and anti-apoptotic markers BCL-2, BCL_{XL} expression in the human endometrial hyperplasia cells treated with 50 and 100 μM genistein. The possible mechanism of genistein induced apoptosis was also examined in endometrial hyperplasial cells. It has been reported that anti-apoptotic BCL-2 family proteins are involved in caspase dependent apoptosis. Results showed that genistein noticeably induced Bim expression. We found that genistein increased the p53 (Fig. 7 A) and active (cleaved) caspase-9 expression in a concentration-dependent manner. The subsequent activation of Mdm2, caspase-3 and PARP were also measured. A significant reduction of anti-apoptotic protein was observed dose-dependently whereas significant induction was observed in the expression of pro-apoptotic protein in human endometrial hyperplasial cells. About ~ 50 % reduction was observed in BCL-2, $BCL_{XL,}$ ($p < 0.001$) expression whereas ~ 45 % upregulation in phosphorylated CREB and ~ 85 % in Bax ($p < 0.001$), ~ 90 % NOXA ,~70% in PUMAα ($p < 0.001$), ~ 90 % in phosphorylated Bad , ~ 75 % in Bim ($p < 0.001$) , ~ 70 % in cleaved caspase-9 , 90 % in cleaved caspase-3 ($p < 0.001$), ~ 80 % in cleaved PARP ($p < 0.001$) protein expression at 100 μM genistein treatment was detected in human endometrial hyperplasial cells (Fig. 5 A, and B). Besides this, genistein also reduced the expression of Mdm2 ~ 45 % ($p < 0.001$) and induced the expression of p53 ~ 60 % ($p < 0.001$) at 100 μM concentration (Fig.7 A). Dose-dependent effects of genistein were also observed on expression of apoptotic markers in human endometrial hyperplasial cells and significant changes were found (Supplementary Fig. 2). However, no significant change was found in expression levels of Bax , BCL-2 proteins in human normal endometrial cells (Supplementary Fig. 3).

Further, we also studied the effect of genistein on the mRNA expression of apoptotic genes by quantitative real time PCR and found, ~ 1.4 and ~0.8 fold induction in Bax and caspase-3

expression respectively whereas the BCL-2 expression was found to be reduced by ~ 0.6 fold at 100 μM concentration (Fig.5 C). These results clearly indicated that the reduction in protein expression levels of apoptotic markers was due to reduced mRNA expression caused by genistein.

Next, the mitochondrial membrane potential (MMP) of hyperplasial cells was analyzed and results showed ~80 % drop in MMP, in the presence of 100 μM genistein ($P>0.001$). These results show that the apoptotic signaling pathway activated by genistein is likely to be mediated via the mitochondrial pathway or intrinsic pathway (Fig.4 B).

3.6. Genistein interferes with the PI3K/Akt survival pathway in endometrial hyperplasial cells

We also studied the effect of genistein on the PI3K/Akt pathway, an important cell survival and proliferation pathway in endometrial hyperplasia development. Phosphorylation status of PI3K (tyr 485) and Akt (ser 473) was significantly downregulated by genistein. The densitometric analysis of immunoblots showed that the genistein decreased the intracellular levels of phosphorylated PI3K by ~ 50 % ($p <0.001$), which in turn decreased the activation of Akt by ~ 55% ($p <0.001$), at a concentration of 100 μM of genistein (Fig. 7 B).

4. Discussion

Genistein is reported to bind to ERα and ERβ, though with higher affinity to ERβ [29], and behaves either as an estrogen agonist or antagonist to one or both ERs depending on genistein dosage and timing of exposure[30]. Endometrial hyperplasia represents a non-physiological, precancerous non-invasive and abnormal proliferation of the endometrium[31]. In this study, we have established a human primary atypical endometrial hyperplasial cell culture for studying

effect of genistein on the human tissue. It was interesting to observe that the genistein significantly decreased the growth of human endometrial hyperplasial cells but not of normal human endometrial cells. While exploring the mechanistic action of genistein, we found that the genistein exerts anti-proliferative activity via reducing EGF binding to EGFR and causing the inhibition EGFR activation as observed in primary human endometrial hyperplasial cells. EGFR and its family members are the major contributors of a complex signaling cascade that modulates proliferation, survival, differentiation, apoptosis, adhesion and signaling of cancer cells. Due to their extraordinary role in the progression of cancer, EGFR and its family members have emerged as attractive agent for cancer therapy[32,33]. The phosphorylated tyrosine 1173 of EGFR can function as a docking site for PI3K/Akt signaling pathway[34]. This is the first study to report the EGFR mediated-growth inhibitory action of genistein on endometrial hyperplasial cells.

The BCL-2 family, has fundamental role in the regulation of apoptosis. This family includes pro-apoptotic proteins (Bax, Bad, Bak, PUMAα, Bim and Bid) and anti-apoptotic proteins (BCL_{XL}, MCL-1 and BCL-2)[35]. It has been demonstrated that proteins of the BCL-2 family are critical death regulators of mitochondrial integrity and its dependent apoptosis. Some of them are governed by PI3K/Akt signaling pathways through translational and post-translational modifications. Bad binds to BCL-2 or BCL_{XL} and inhibits their anti-apoptotic potential. We have found that the genistein induced the expression of pro-apoptotic markers at mRNA and protein level, in endometrial hyperplasial cells. Also genistein induced the activation and cleavage of caspase-9, caspase-3 and caused a significant drop in mitochondrial transmembrane potential (MMP) indicating involvement of intrinsic pathway, in genistein-induced apoptosis in endometrial hyperplasial cells. Genistein, in particular, is recognized as an inhibitor of tyrosine-specific protein kinase[36], and induces apoptosis in human colon cancer cells through inhibiting NF-κB pathway[5]. EGFR blockade alone may not

be sufficient for the control of growth of human endometrial hyperplasial cells because of the independent activation of Akt and NF-κB. The transcription factor NF-kB is a regulator of genes encoding cytokines and cytokine receptors molecules that drive immune and inflammatory responses. NF-kB is involved in both proliferation and apoptosis via anti-apoptotic genes BCL-2, BCL_{XL} and XIAP, growth inducible ErbB2 and cell cycle regulating gene cyclin D1[37-40]. Because NF-kB has a central role in the regulation of survival and apoptotic pathways, we measured the effect of genistein on expression of the NF-kB in endometrial hyperplasial cells. Genistein inhibited the activation of PI3K and Akt which led to inhibition of activation of downstream effector, NF-kB. Further, it was interesting to note that genistein significantly increased the expression of ERβ protein besides increasing apoptotic signals which are known to be functionally involved in the rhythmic proliferation and differentiation of human endometrium[41]. Also, genistein suppressed p-Akt and induced the activation of p53. The transcription factor p53 is known to activate genes involved in apoptosis, DNA repair, growth arrest, and angiogenesis[42]. Altogether, these data suggest that genistein significantly down regulates the EGFR and PI3K/Akt/NF-κB signalling which might be considered as one of the mechanisms responsible for specific apoptotic and anti-proliferative activity of genistein in human endometrial hyperplasial cells.

5. Conclusion

To conclude, genistein inhibits cell proliferation through discontinued EGFR signalling, and induces apoptosis in primary endometrial hyperplasial cells via inhibiting the cell survival pathway PI3K/Akt and NF-κB. Thus, our findings have helped elucidate the mechanisms by which genistein may contribute to the prevention of endometrial hyperplasia.

Acknowledgments

We thank Mr AL Vishwakarma, Dr K Mitra, and Dr Kavita Singh, SAIF-facility, CSIR-CDRI for help in flow cytometric analysis and confocal microscopy. Financial support was provided by Council of Scientific and Industrial Research (CSIR) and Indian Council of Medical Research (ICMR), New Delhi. This is CDRI communication number X2/2015-AD.

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Figure legends

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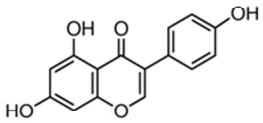

Genistein

(4′,5,7-Trihydroxyisoflavone, 5,7-Dihydroxy-3-(4-hydroxyphenyl)- 4H-1-benzopyran-4-one)

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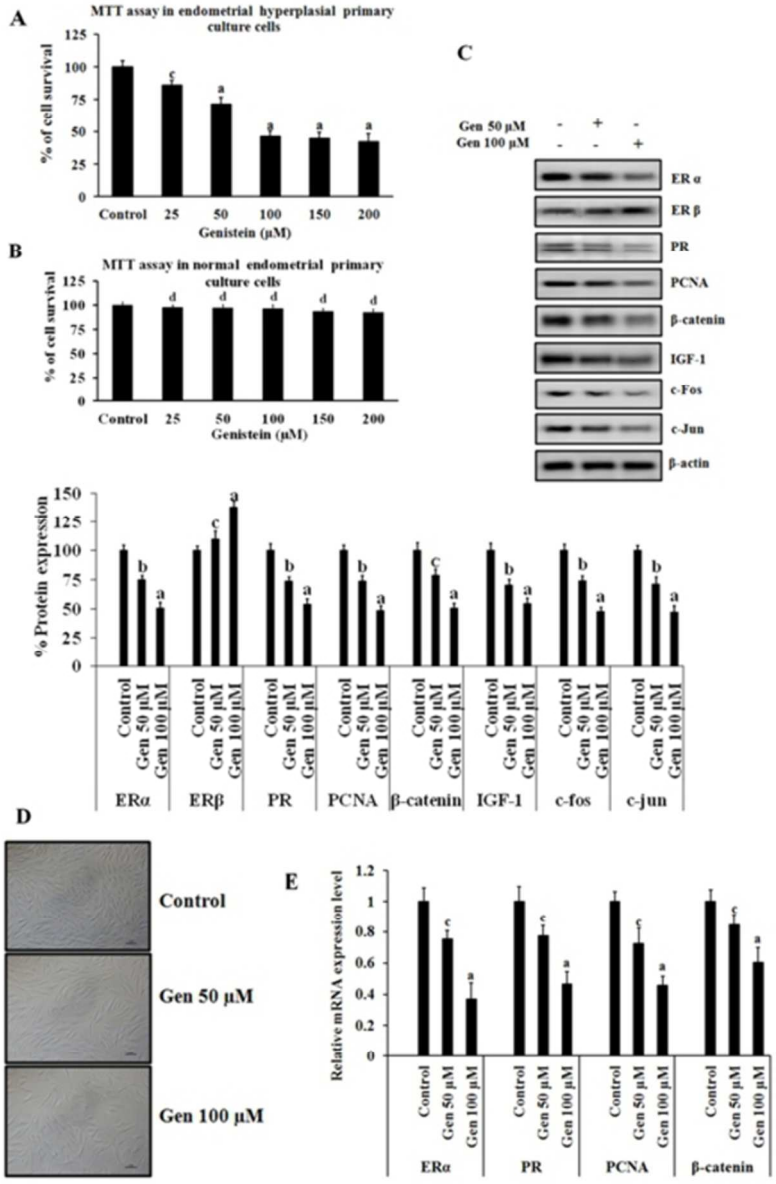

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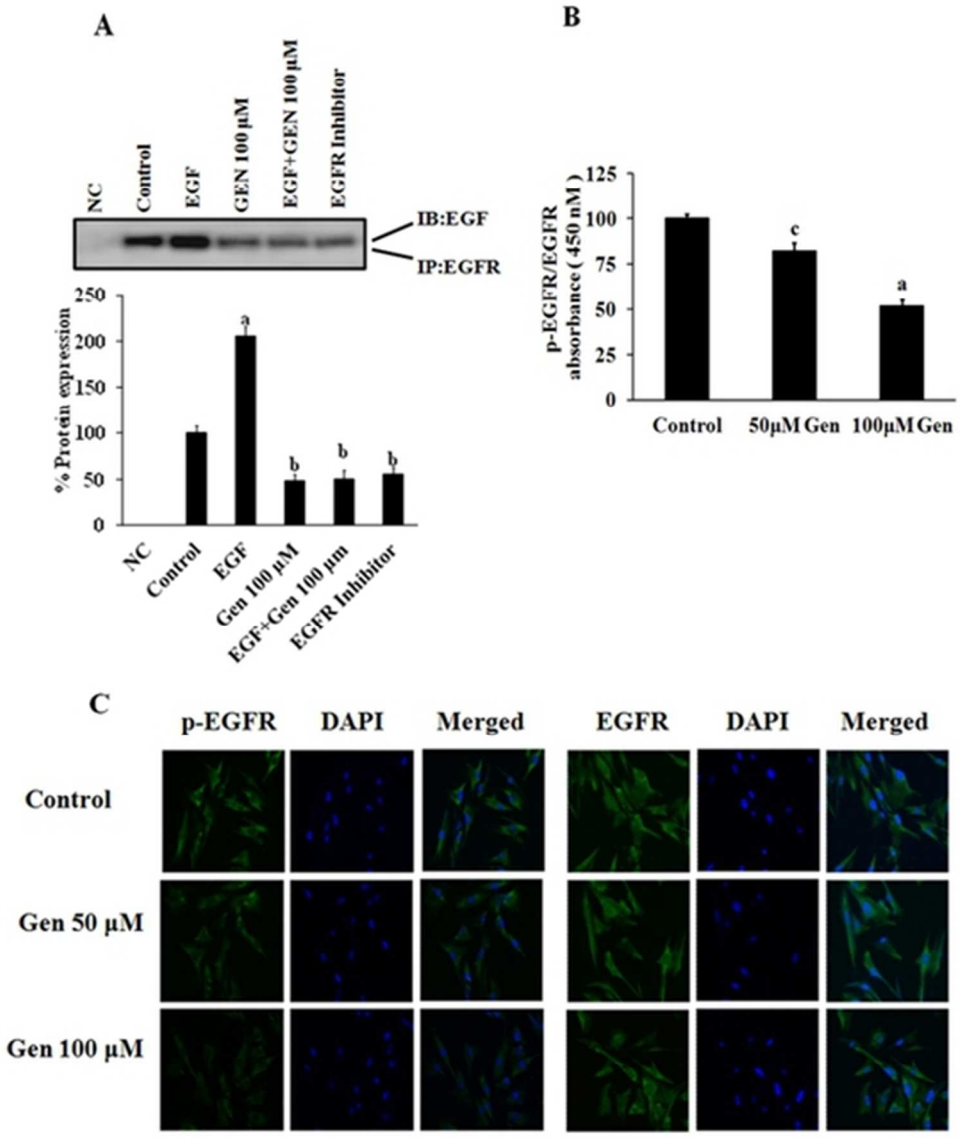

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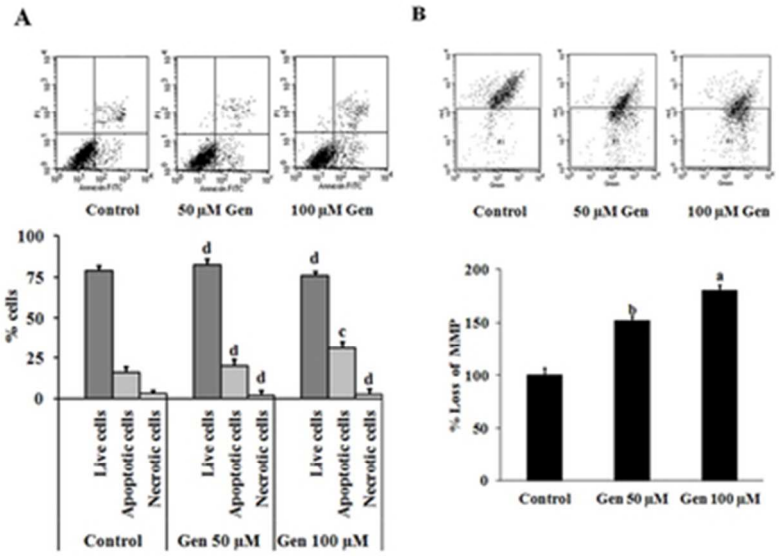

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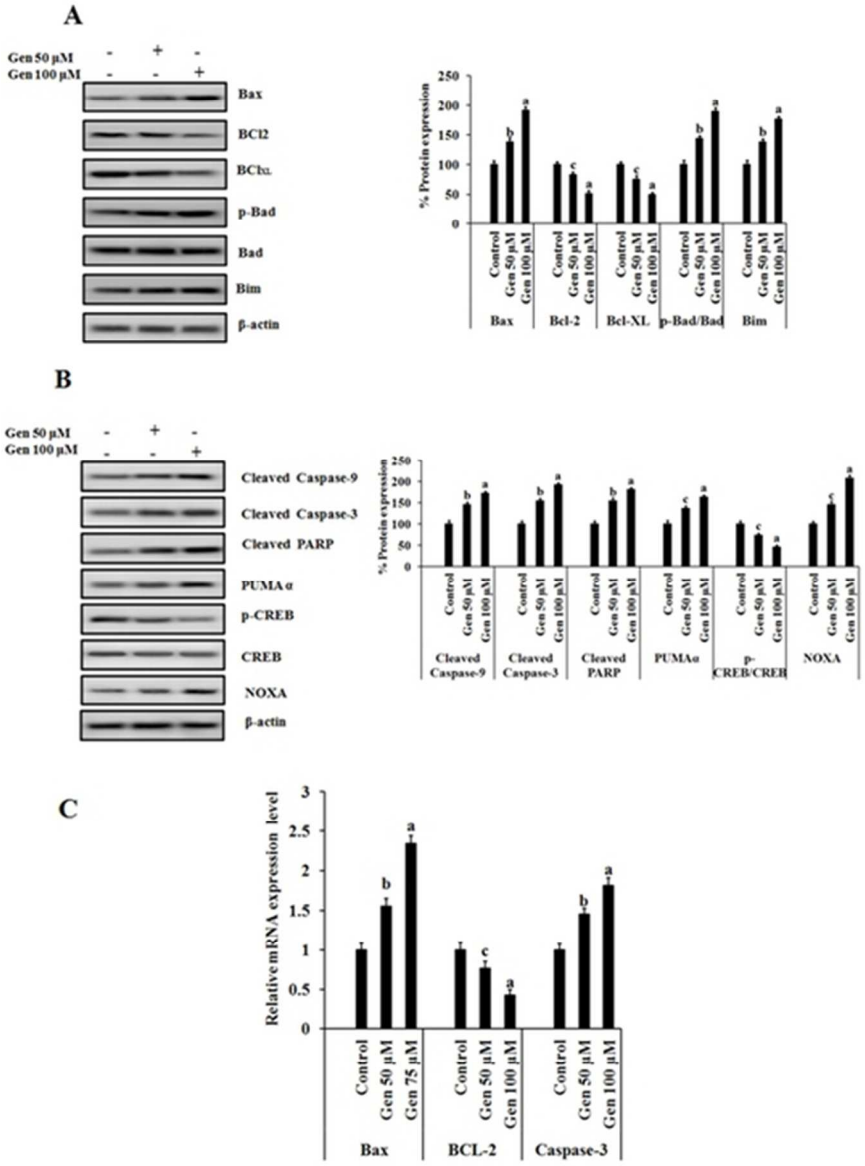

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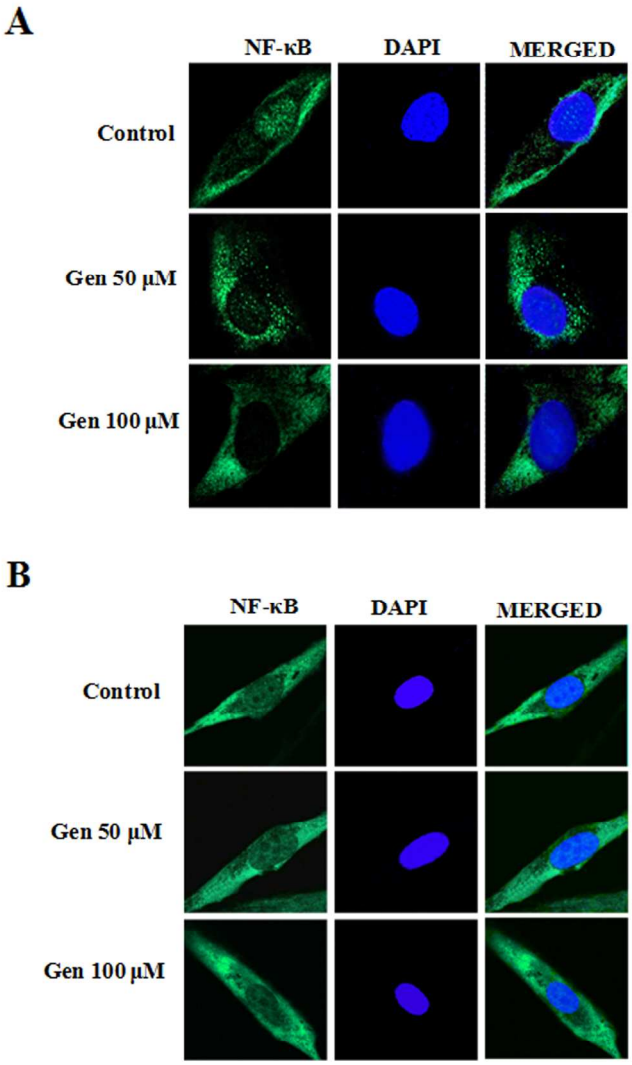

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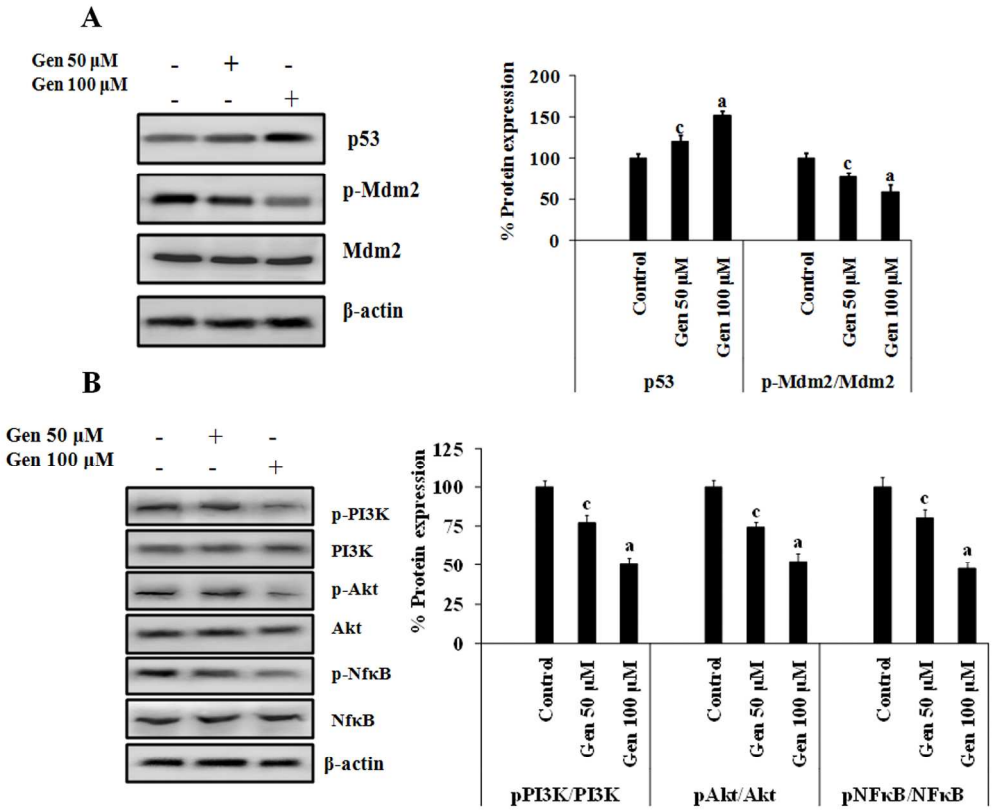

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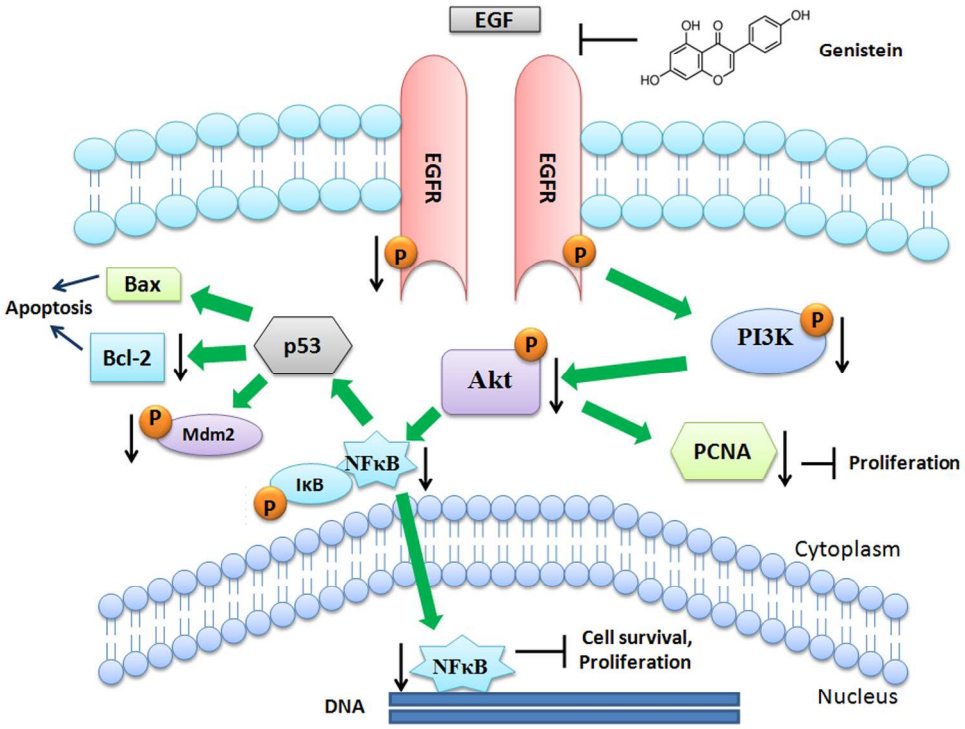

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