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ORIGINAL ARTICLE

Degalactotigonin Inhibits Invasion and Induce Apoptosis by Targeting TGF-β Signalling in Oral Cancer Cells

Yang Li[1], Chengnan Gu[2]

[1]Department of Orthodontics, Daqing Oilfield General Hospital, Daqing, Heilongjiang, 163001, [2]Department of Stomatology, Nantong Haimen People's Hospital, Nantong, Jiangsu, 226100, China

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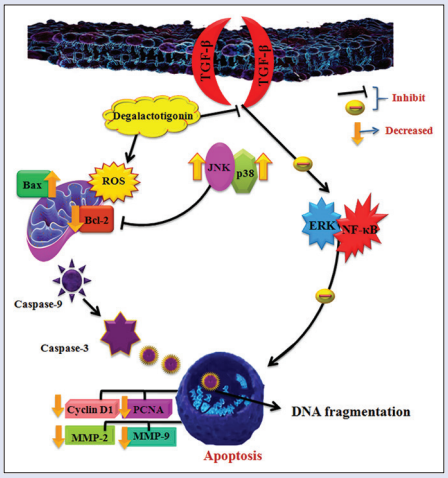
ABSTRACT

Background: Overexpression of transforming growth factor-β (TGF-β) and its cancer regulators are the major phenomena in oral cancer. The study aimed to investigate the anticancer effect of degalactotigonin (DGT), a steroidal glycoside from *Solanum nigrum* on oral cancer. **Objectives:** The study aimed to investigate the anti-invasion and apoptotic induction effect of DGT in oral squamous cell carcinoma (OSCC) cells through inhibiting non-canonical TGF-β signalling. **Materials and Methods:** Human oral cancer KB (KERATIN-forming tumor cell line HeLa) cells were chosen to study the anti-cancer activity of DGT *in vitro* experiments. The cytotoxic effect of DGT was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. Cell growth, DNA damage, invasion inhibition and apoptosis activation were evaluated by mitochondrial membrane potential (ΔΨM), comet assay, reactive oxygen species (ROS) and AO/EtBr staining. The DGT effect on protein expression levels related to invasion, apoptotic markers and its activation signalling pathways on KB cells were examined by western blotting. **Results:** We found that DGT antioxidant properties reduced cell viability, generates ROS, enhanced DNA damage, and MMP dissipation. Further, TGF-β inhibitions resulted in a reduction of extracellular signal-regulated kinase (ERK), NF-κB and activation of JNK, p38 which increase ROS in a cancer cell that downregulates cyclin-D1, PCNA, MMP-2, MMP-9, Bcl-2 and increases Bax, Caspase-9, Caspase-3 protein expressions that simultaneously subdues the cancer developments. **Conclusion:** These findings suggest that DGT inhibiting TGF-β mediated ERK, NF-κB and activation of JNK, p38 caused tumour cell death via ROS stimulation and apoptosis.

Key words: Degalactotigonin, ERK, invasion and apoptosis, NF-κB, oral cancer, TGF-β

SUMMARY

- DGT inhibits oral squamous cell carcinomas by induce ROS mediated cell death.
- DGT invasions by subdued MMP-2 and MMP-9 expression and activate apoptotic Bax signalling.
- DGT treatment suppress tumour progression by abrogates ERK phosphorylation and NF-κB nuclear translocation through inhibit proto-oncogene TGF-β signalling.



Abbreviations used: ROS: Reactive oxygen species; MMP: Mitochondrial membrane potential; TGF-β: transforming growth factor- β; MMP-2: Matrix metalloproteinase-2; MMP-9: Matrix metalloproteinase-9; JNK: c-Jun N-terminal kinase; Bcl-2: B-cell lymphoma 2; Bax: Bcl2-associated X protein; Caspase-9: Cysteine-aspartic protease-9; Caspase-3: Cysteine-aspartic protease-3; NF-κB: Nuclear factor-κB; ERK: Extracellular signal-regulated kinase.

Correspondence:

Dr. Chengnan Gu,
Department of Stomatology, Nantong Haimen People's Hospital, Nantong, Jiangsu - 226100, China.
E-mail: ntgcn8080@sina.com

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) includes malignant squamous cell carcinoma that occurs in the mouth, oropharynx, or larynx. It is considered the sixth most common cancer in the world.[1] Nearly 539,575 new cases were diagnosed and about reached 268,362 deaths are expected in 2020.[2] Despite advancements in diagnosis and recovery treatment, the 5-year survival rate remains low for OSCC patients and is still unsatisfactory, which is 50%–60%.[3] Several prognostic factors have been identified, including smoking and alcohol consumption, but the mechanism of OSCC carcinogenesis remains elusive.

Sustained invasion is a hallmark of cancer. Matrix metalloproteinases (MMPs) are typical enzymes that have been primarily involved in migration, in particular, MMP-2 and MMP-9 promote tumor cells to invade normal tissues and spread to other organs when subject to degradation of extracellular matrices.[4] As a result, MMP-2 and MMP-9 are well recognised as markers of poor prognosis and have been identified in the majority of OSCC-affected

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individuals,[5] which represents these major target proteins in cancer treatment. Over-activated signal transduction pathways alter many of the cell biological features that are beneficial to cancer cell-dependent and invasion markers.[6]

The upstream oncogenic signalling receptor transforming growth factor-β (TGF-β) is the major participant that is deeply involved in the progression of different types of cancers.[7] In a malignant environment, TGF-β acts as the primary stimulant of tumour growth, invasion and immune development, and suppression of apoptosis. Recent studies further proved that non-canonical TGF-β signals are transduced mainly through the oncogenic pathways such as MAPKs.[8] Activation of TGF-β receptor tyrosine kinase signalling stimulates ERK and NF-κB, which can increase active proliferative, and invasive factors and downregulate apoptosis that is allowing the cells to become invasive and vascular growth.[9] Therefore, conquering TGF-β and its downstream signalling in a malignant environment is an ideal strategy for tumour intervention. However, the drugs targeting oncogenic TGF-β function are still under extensive investigation.

Natural phytochemicals have popularly exhibited their chemopreventive properties by modulating signalling involved in uncontrolled cell growth, invasion, and apoptosis in malignant cells.[10] Degalactotigonin (DGT), a phytochemical isolated from *Solanum nigrum* has antioxidant, anti-inflammatory, and anti-tumour effects.[11] A recent report has proposed that DGT exists inhibits GSK3β mediated metastasis of Osteosarcoma while normal cells are unaffected.[12] Tuan Anh *et al.*[13] revealed that DGT treatment cause arrests in the cell cycle and induces apoptosis via suppression of the EGFR signals in pancreatic cancer cells. However, the molecular determinants of DGT remain poorly understood in oral cancer. Therefore, we investigated the anticancer effect of DGT by suppressing invasion and inducing apoptosis by inhibiting TGF-β signalling in KB human oral cancer cells.

MATERIALS AND METHODS

Material

Trypsin EDTA, Phosphate Buffered Saline (PBS), Acridine Orange (AO), Dulbecco's Modified Eagles Medium (DMEM), Ethidium Bromide (EtBr), 2-mercaptoethanol, Rhodamine 123, Hoechst 33342, 2,7-diacetyl dichlorofluorescein (DCFH-DA), glutamine- penicillin-streptomycin solution (antibiotics), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Hi-media. DGT, monoclonal anti-bodies TGF-β, Bcl-2, ERK, p-ERK $^{(Thr202/Tyr204)}$, JNK, p-JNK $^{(Thr183/Tyr185)}$, p38, NF-κB, Caspase-9, MMP-2, Caspase-3, MMP-9, Bax, and IgG-HRP polyclonal antibody were obtained from Sigma Chemicals Co., St. Louis, USA.

Cell culture

The human oral KB cancer cells were received from the Beijing Institute for Cancer Research (Beijing, China) and cultured in Dulbecco's Modified Eagles Medium (DMEM) with fetal bovine serum (10%), 100 U/mL penicillin G, and 100 µg/mL streptomycin at humidified atmosphere with 37°C temperature containing 5% CO_2 environment.

Cytotoxic assay and cell viability

The viability of KB cancer cells was assessed via MTT test studies. In brief, cells were seeded and pre-cultured for 24 h in a 96-well plate, then treated with 0–70 µM of DGT for 24 h. After the cells were incubated with MTT for 4 h, the media was discarded and 100 µL of DMSO was added for dissolving the formazan crystals.[14] Finally, absorbed at 570 nm using a microplate reader (Bio-Rad).

Determination of intracellular ROS generation

The 2,7-diacetyldichlorofluorescein (DCFH-DA) staining was used to measure intracellular ROS secretion in KB cells.[15] Cells were plated (1×10^6 cells/well) and incubated overnight to attach to six well plates. After being treated with DGT then cells were incubated for 24 h, then cells were stained with DCFH-DA and allowed 10 min incubation at 37°C. Measurement of fluorescent was made using Shimadzu RF-5301 PC spectrofluorometer with filters set excitation at 485 ± 10 nm and emission at 530 ± 12.5 nm, respectively. A fluorescence microscope (Nikon, Eclipse TS100, Japan). with a blue filter (450–490 nm) was utilized to further observe the cells.

Observe mitochondrial membrane potential (MMP) loss

MMP (DCM) was assessed by staining with the lipophilic cationic dye Rhodamine-123 (5 mM).[16] The cells were cultured in six well plates (1×10^6 cells/well) and then incubated with DGT. A plate with six wells were used to seed (1×10^6 cells/well) the cells and then incubated with DGT for 24 h and then cells were kept for 30 min incubation with Rh-123 dye. After, cells were rinsed with PBS and observed using a fluorescence microscope with peak intensity at 450–490 nm. The fluorescence intensity was measured with an excitation filter of 485 ± 10 nm and an emission filter of 530 ± 12.5 nm.

Determination of apoptotic morphological changes

The KB cells were stained with acridine orange and ethidium bromide (AO/EtBr) to detect apoptotic cell confirmation.[17] Concisely, 3×10^4 cells/well concentrations were cultured on a six-well plate with DGT treatment for 24 h. After cells were predetermined on methanol with glacial acetic acid at 3:1 concentration and incubated at 4°C for 30 min, then rinsed with PBS, and 1:1 proportion of AO/EtBr stained for 30 min at 37°C. The fluorescence microscope was utilized to reveal the number of apoptosis cell features and count the total number of cells that function in the present field.

Analysis of DNA damage (comet assay)

The treated and untreated KB cells were harvested and mixed with 120 µl of 0.5% low melting point agarose.[18] Add the mixture to a 1% normally melted agarose on glass slide, and immerse it at 4°C (10% DMSO, Tris pH 10, 100 mM Na 2 EDTA, 1% Triton X-100, 2.5 M NaCl) solution. After DNA denaturation, transfer the slides to a horizontal system for gel electrophoresis and pour the fresh running buffer into a solution with a pH of 13, The treated and untreated KB cells were harvested and mixed with 120 µl of 0.5% low melting point agarose. Rinse the slide with neutralization buffer (Tris 0.4 M, pH 5) three times and stain with 20 µg/mL ethidium bromide for 15 min, and then store in the dark to determine DNA damage. Equipped with an epifluorescence microscope Nikon with 40x objective lens, (Japan Eclipse TS100), equipped with 510–560 nm excitation filter and 590 nm Nikon 4500 Coolpi blocking filter; Digital camera (Japan) was used to capture comet images and analyzed with CASP software.

Immunoblot analysis

The harvested DGT-treated cells were rinsed with ice-cold PBS and lysed with buffer (RIPA) for 30 min. The protein content from each cell extract was fractionated by 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and then transported to the PVDF membrane. Block the membrane through BSA and probe with monoclonal antibody and incubate overnight at 4°C. Eventually, the

membrane was treated with appropriate secondary antibodies for 1 h then rinsed two to three times via PBST, and finally detected with a chemiluminescent detecting system.

Molecular docking

The three-dimensional structure of proteins TGF-β-RII and ERK were downloaded and studied structural information from Protein Data Bank and Uniprot. Schrodinger software was used for predicted binding sites based on Sitecore and D. Further PubChem database was used to download DGT. protein preparation, receptor grid generation, and ligand preparation methods were utilized for ligand-protein docking. The formation of disulfide bonds, the addition of missing hydrogen atoms, the assignment of bond sequences, and the orientation of hydroxyl and amide groups in amino acids are all performed by the protein preparation wizard. The OPLS3e force field minimizes protein energy, and non-hydrogen atoms are minimized until the average root mean square deviation (RMSD) reaches the target value of 3Å. Ligprep tool applies for ligand preparation, OPLS3e was fixed for force field and ionized, Epik use for produce perfect structure and docking of ligand executed by XP (extra precision) docking method.

Statistical analysis

ANOVA on SPSS 11.0 software package was used for statistical analyses. Further, Duncan's multiple range test (DMRT) was used to analyze significant differences ($P < 0.05$) between the means.

RESULTS

Effect of DGT on the viability of cell

We observed the inhibitory effects of 50 (IC_{50}) of DGT in different dosages (0, 10, 20, 30, 40, 50, 60, and 70 μM) in KB cells through an MTT study. Maximum cell death was observed at 60 μM. Hence, DGT IC_{50} level 30 μM apparent from the growth inhibition curve and we selected 10, 20, and 30 μM dosages of DGT for further studies [Figure 1].

Effect of DGT on ROS generation

We measured the formation of intracellular ROS after exposure to DGT in KB cells by staining with DCFH-DA. Increased green fluorescence intensity shows substantially increased ROS production of DGT (10, 20, and 30 μM) treated KB cells [Figure 2 aii–iv] as assessed to the untreated cells [Figure 2 ai].

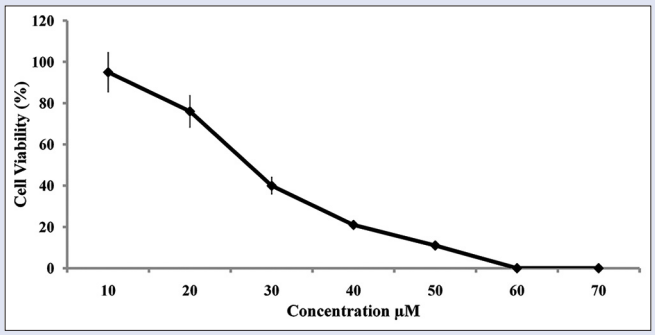

Figure 1: Effect of DGT in KB cell viability assessed using MTT assay. (a) Inhibition of cell growth is the effect of DGT on KB cells. Values are reported as the mean ± SD of each group of six experiments

Effect of DGT on MMP

We explored the MMP that was stimulated by apoptosis in KB cells added by DGT at different concentrations (10, 20, and 30 μM). The significant loss of MMP (green intensity) was observed in a DGT dose-dependent manner [Figure 3 aii–iv] as compared to untreated cells [Figure 3 ai].

Effect of DGT on apoptotic morphological changes

The enhanced apoptosis activity of DGT in KB cells was measured by AO/EtBr staining. The EtBr red fluorescence entered particularly into apoptotic nuclei, whenever AO green dye reached up only by healthy cells. In our study, DGT (10, 20 and 30 μM) treated [Figure 4 aii–iv] cells established orange colour as untimely apoptotic and delayed apoptosis as in red, while bright green nucleus fluorescence present in control suggests viable cells [Figure 4 ai].

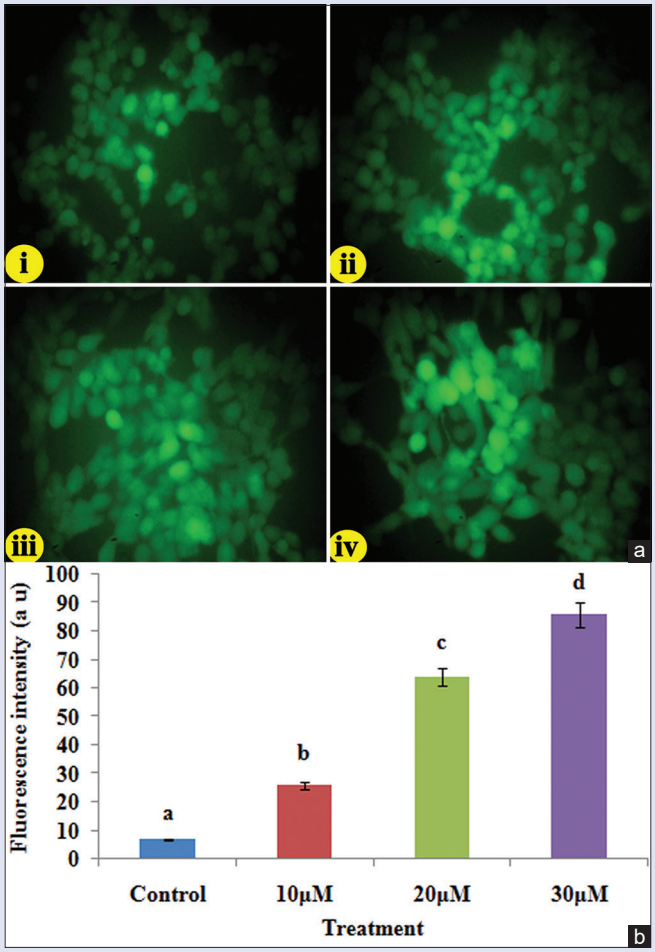

Figure 2: Effect of DGT on the formation of intracellular ROS in KB cells used to determine the DCFH-DA staining method. (a) Micrographs revealed that (a i) untreated KB cells showed weak DCF fluorescence. (a i-iv) Various concentrations (10, 20, and 30 μM) of DGT showed increased formation of ROS, indicating deep DCF fluorescence intensity. The image was taken by the floid cell imaging station. (b) Measure the percentage of ROS formation with a spectrofluorometer. All the experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (mean ± SD). An analysis of variance followed by DMRT was used to determine statistical significance. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

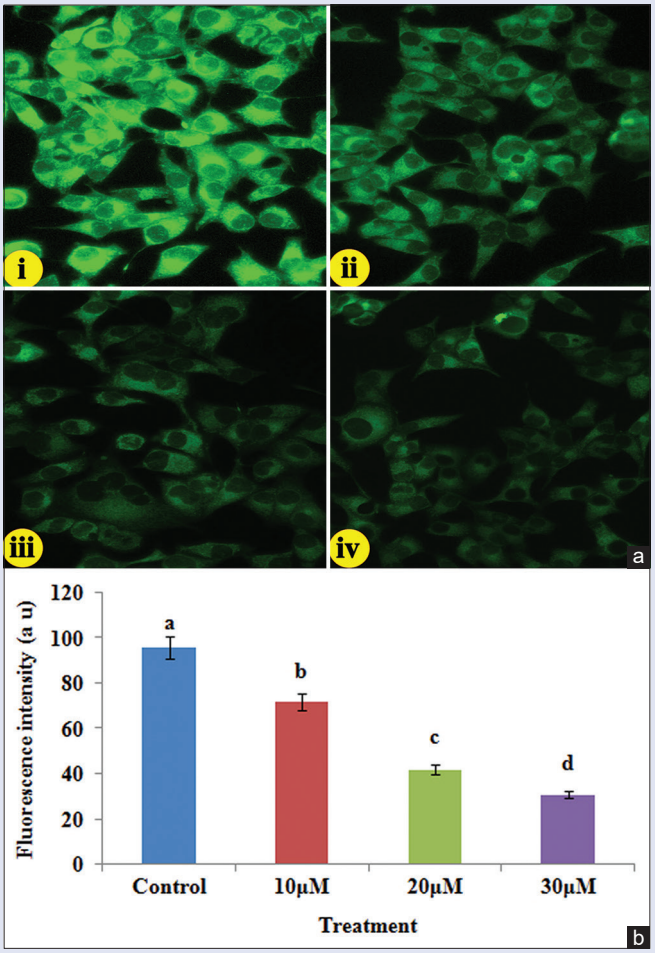

Figure 3: Evaluate the effects of DGT on MMP in KB cells to utilize Rhodamine 123 staining. (a) (a i) The high fluorescence was observed in untreated KB cells, which indicates polarization of the mitochondrial membrane. DGT (a ii–iv) various concentrations (10, 20, and 30 μM) within 24 h indicate collapse of the mitochondrial matrix. The image was taken by the floating cell imaging station. (b) The intensity of the fluorescence was displayed through the detection of spectrofluorometer. All the experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (mean ± SD). An analysis of variance followed by DMRT was used to determine statistical significance. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

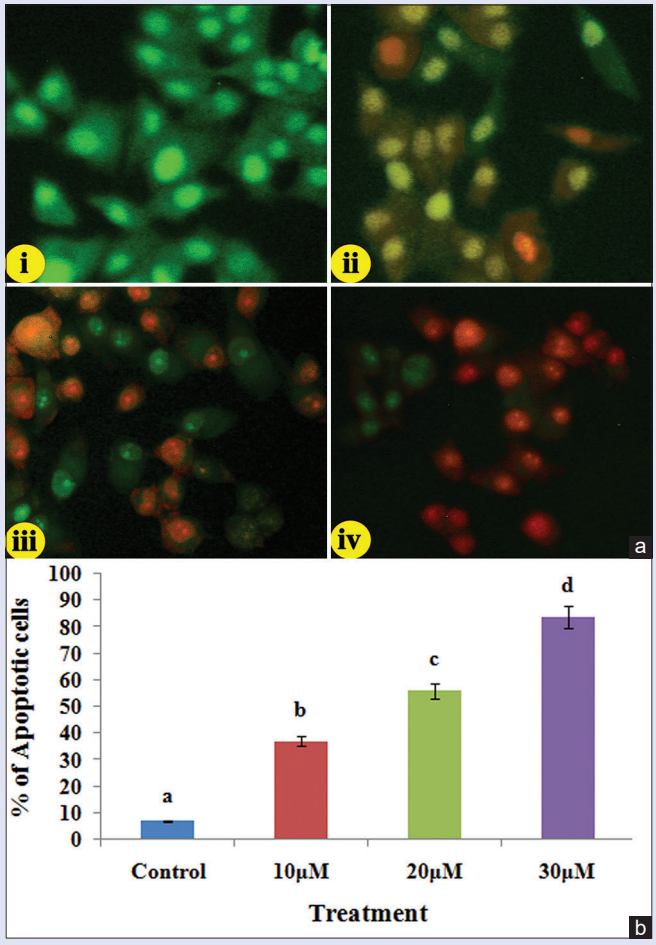

Figure 4: The effect of DGT on the morphology of cell apoptosis was detected by dual staining (AO/EtBr). (a) (a ii–iv) DGT at various concentrations (10, 20 and 30 μM) showed an increase in the apoptotic cells percentage compared to (a i) untreated KB cells, (b) reveal that percentage of apoptotic cells is determined. Data are expressed as the three independent experiments mean standard deviation (SD). All the experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (mean ± SD). An analysis of variance followed by DMRT was used to determine statistical significance. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

Effect of DGT on DNA fragmentation

To determine DGT DNA damage effects on KB cells that were observed by comet assay. The DNA damage was significantly increased by DGT different (10, 20, and 30 μM) concentrations [Figure 5 aii–iv] by comet test parameters such as % of head DNA, length, moment tail, olive tail moment (OTM), respectively. Untreated cells [Figure 5 ai] have the circle and non-fragmented intact nucleoid indicating DGT fragmentation effects.

Effect of DGT on invasion protein expression

To examine the molecular mechanism of DGT (10, 20 and 30 μM) on inhibition of proliferative and invasive proteins by western blot studies. As shown in Figure 6, DGT significantly reduced the cell proliferative and invasion markers MMP-2, and MMP-9 protein expression on KB oral cancer cells concentration wise. These results propose that DGT modulatory effect on cancer cells.

Effect of DGT on promoting apoptosis

We further determined the apoptosis activity of DGT (10, 20 and 30 μM) and whether reduction of anti-apoptotic and stimulation of pro-apoptotic markers expression in KB oral cancer cells. Compared with control, DGT suppresses Bcl-2 expression, which increases levels of Bax, Caspase-9, and Caspase-3 expression in these cell lines. These results suggest apoptosis activity of DGT via downregulating growth factor and Bcl-2 response [Figure 7].

Effect of DGT on TGF-β signalling

TGF-β dependent phosphorylation and transcription factor activation is a well-known key mechanism responsible for the invasion and diminution of apoptosis. In the current study, we found increased expression of TGF-β, ERK, p-ERK, NF-κB and reduction of JNK, p-JNK, and p38 on control cells. DGT (10, 20 and 30 μM) treated the cells establish significantly suppress TGF-β, ERK, p-ERK, and NF-κB with upregulated JNK, p-JNK, p38 protein expression [Figures 8 and 9].

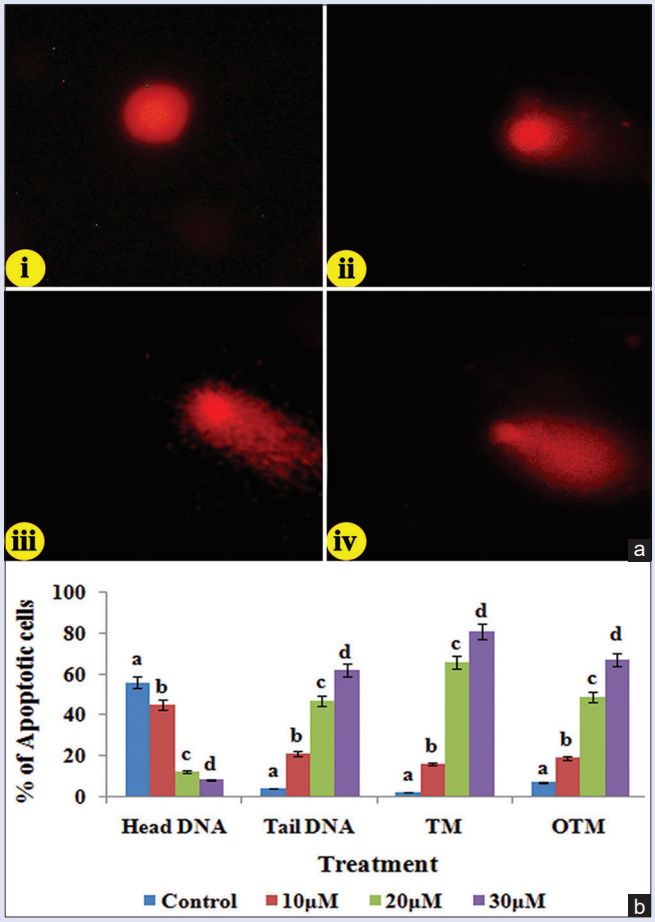

Figure 5: Effect of DGT on the single stand break of DNA in KB cells. The cell lysates were electrophoresed, and break occurred in DNA molecule two liner strands were determined by ethidium bromide staining using single-cell gel electrophoresis. (a) (a ii–iv) significant changes have been observed in comet figure of cells have been treated with DGT various concentration (10, 20 and 30 µM) as compared (a i) untreated KB cells. (b) The results showed that DNA damage was characterized by the head DNA percentage, DNA tail, moments of the tail, as well as movement of olive tail in cells treated with degalactotigonin. All the experiments were carried out in triplicate, and the data were expressed as mean ± standard deviation (mean ± SD). An analysis of variance followed by DMRT was used to determine statistical significance. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

These results indicate that DGT inhibits invasion and activates apoptosis in oral cancer cells by hindering TGF-β/ERK/NF-κB.

Molecular docking

The active site of TGF-β-RII and ERK in the binding pockets was docked with DGT. The docked DGT complex has hydrogen bond interaction LYS-252, ARG-254, LYS-381, ARG-423 with TGF-β-RII at a distance of 1.61, 2.99, 1.83, 2.61 Å [Figure 10a], TYR-36, LYS-151, ASN-154, ASP-167, with ERK at a distance of 2.22, 2.46, 2.03, 2.33 Å [Figure 10b], respectively. Therefore, DGT good interactions can inhibit the expression of TGF-β and ERK-mediated transcription.

DISCUSSION

Inhibition of invasion and promoting apoptosis are handling a double-edged sword in chemotherapy due to numerous transcription factors and upstream signals majorly implicated in those functions. Traditional chemotherapy drugs have limitations for several side effects on patients with cancer owing to damage normal cells.[19,20] Hence novel agents can exert a palliative effect that inhibits oncogenic signals and transcription factors, as well as less toxic to normal cells, which is urgently needed. Many phytochemical antioxidant systems being extensively explored anticancer results with less or no toxicity to normal cells could be developed as alternative medicine in chemotherapy.[10] Saponins possess potent anticancer effects on tumour cells originating in various organs by interfering with the replication of cellular DNA, cell cycle arrest, and inducing apoptosis.[21] In this study, we analyzed that steroid saponin DGT could act as a proliferative and invasion inhibition via modulating the transforming growth factor (TGF-β) signals and thus enhance the apoptosis effect on the human oral cancer KB cells.

ROS is an intrinsic stimulus that instantly promotes cell cycle arrest and apoptosis. Elevated levels of ROS production induce oxidative stress leading to DNA damage, and impaired MMP thus accelerating the apoptotic cascade. Hence, ROS modulators have recently been proposed as a treatment strategy for cytotoxic to growing cells and specific for targeting cancer cell destruction.[22] We observed DGT treatment stimulated the ROS generation in KB cells, resulting in damaged DNA, loss of MMP, and cellular deterioration which induce apoptosis and ensue cancer cell death. Consistent with our findings, protodioscin a steroid saponin that induces apoptosis through ER stress caused by ROS in human cervical cancer (HeLa and C33A) cells.[23] Besides, corosolic acid declined the potential of the mitochondrial membrane and causes DNA damage and apoptosis in human hepatocarcinoma (BEL-7402, BEL-7404, and SMMC-7721) cells, which were concomitant with our findings.[24]

Increased cell migration and invasion are considered crucial requirements of cancer progression. Among matrix metalloproteinase enzymes MMP2 and MMP9 are extremely involved in tumour invasion, and angiogenesis via extracellular matrix (ECM) degradation. Therefore, therapeutic strategies suppressing MMP2 and MMP9 can lead to tumour invasion and metastasis to other tissues and improve the survival rate of OSCC patients. Several oral cancer research has shown an association between TGF-β and MMPs.[25,26] Treatment with DGT significantly suppresses MMP2 and MMP9, which prevent the invasion and recover ECM in KB cells. Concomitant with the results, the steroidal saponin diosgenin inhibits prostate cancer PC-3 cell invasion by suppressing MMP2 and MMP9.[27] Moreover, solamargine prevents hepatocellular carcinoma (HepG2) cell migration by suppressing the expression of matrix metalloproteinase, which strongly supported our study.[28] Increasing evidence reveals that saponins inhibit invasion are induced apoptotic death by interruption of upstream signalling and transcription factors activate.[29,30] Based on the result, DGT treatment is proved that markedly increase apoptosis through up-regulating Bax and reduction in Bcl-2 expression. Bax releases cytochrome C and stimulates caspase 9 and 3 after the Bc-2 reduction, which results in cancer cell death. These findings could be correlated with Tuan Anh *et al.*[13] who reported that DGT stimulates apoptosis by inhibiting EGFR signals in pancreatic (PANC-1 and MIA-PaCa2) cancer cells. Liu *et al.*[31] revealed that asparanine A induces apoptosis to stimulation of human hepatocellular carcinoma (HepG2) cell cycle arrest. Taken together, it can be speculated that DGT enhances apoptosis in OSCC by inhibiting oncogenic signals.

The invasion markers promoter region contains the transcription factors regulatory element, including ERK and NF-κB. Thus, we studied the inhibition efficacy of DGT on ERK and NF-κB protein expression, which function as primary roles in the invasion. The ERK and NF-κB over-activation negotiate uncontrolled growth and the invasion that

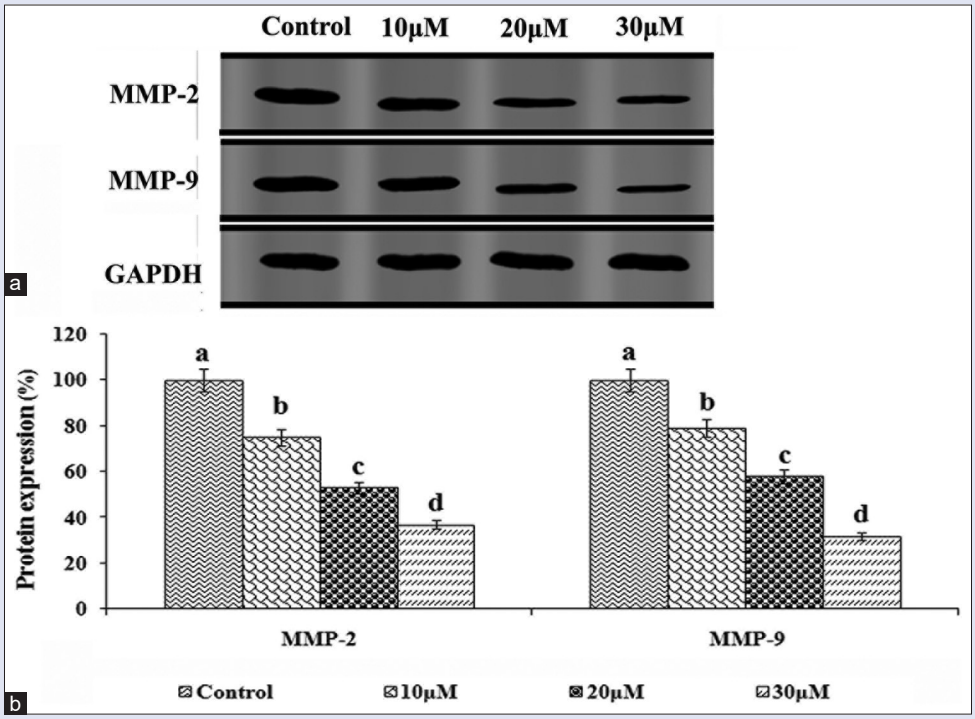

Figure 6: Effects of DGT on the expression of proliferation and invasion. (a) Representative immunoblot analysis MMP-2 and MMP-9 expression after 24 h treatment with and without DGT in KB cells. The loading control protein is GAPDH. (b) Densitometric analysis. The control lysates protein expression was measured six times and was designated as 100% in the graph. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

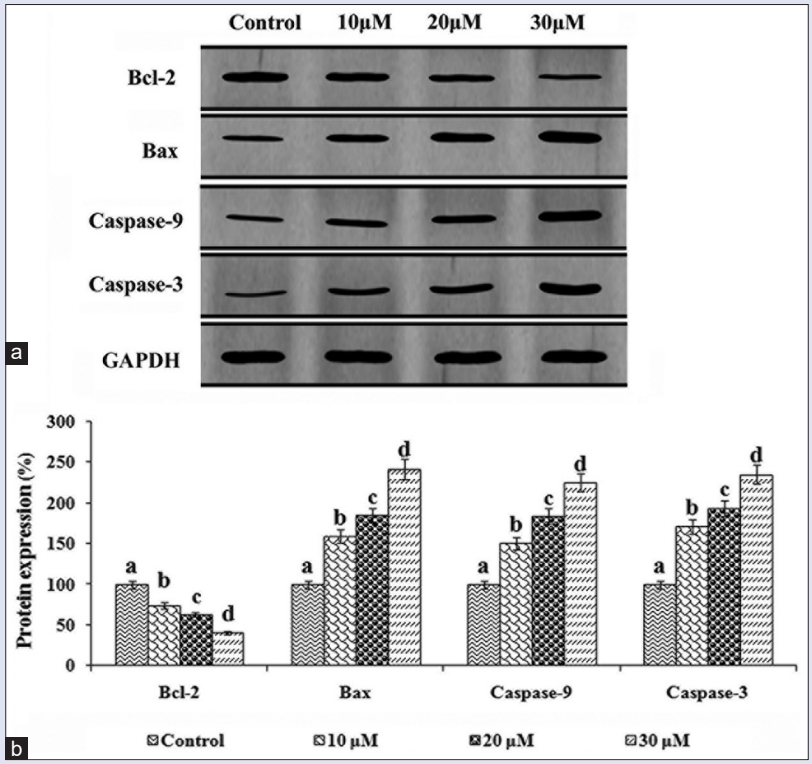

Figure 7: The effect of DGT on the expression of the apoptotic protein. (a) Representative immunoblot analysis of Bcl-2, Bax, cytochrome C, Caspase-9, Caspase-3 and PARP expression after 24 h treatment with and without DGT in KB cells. The loading control protein is GAPDH. (b) Densitometric analysis. The control lysates protein expression was measured six times and was designated as 100% in the graph. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

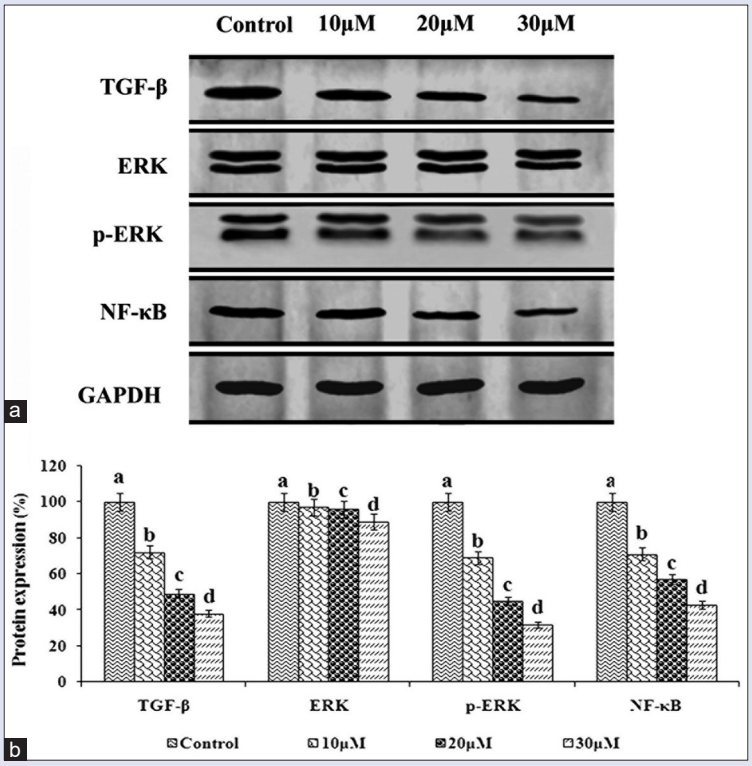

Figure 8: Effects of DGT on TGF-β/ERK/NF-κB protein expression. (a) Representative immunoblot analysis of TGF-β, ERK, p-ERK (Thr202/Tyr204) and NF-κB expression after 24 h treatment with and without degalactotigonin in KB cells. The loading control protein is GAPDH. (b) Densitometric analysis. The control lysates protein expression was measured six times and was designated as 100% in the graph. Values that are not sharing common superscript characters (a–d) that essentially unique at $P < 0.05$ vs. control (DMRT)

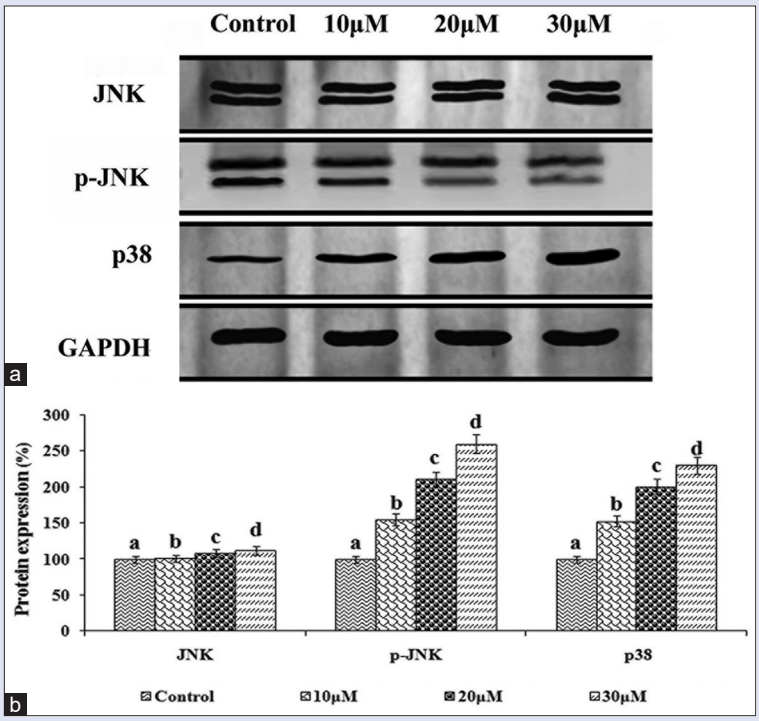

Figure 9: Effects of DGT on JNK, and p38 protein expression. (a) Representative immunoblot analysis of JNK, p-JNK (Thr183/Tyr185) and p38 expression after 24 h treatment with and without DGT in KB cells. The loading control protein is GAPDH. (b) Densitometric analysis. The control lysates protein expression was measured six times and was designated as 100% in the graph. Values that are not sharing common superscript characters (a-d) that essentially unique at $P < 0.05$ vs. control (DMRT)

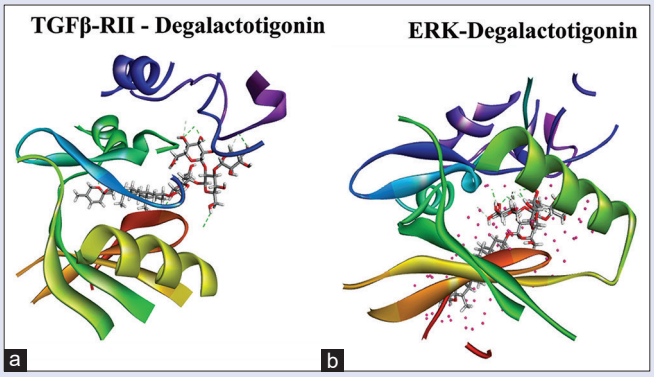

Figure 10: A molecular docking study found that DGT has a strong interaction with residues in the active site of (a) TGFβ-RII and (b) ERK

arises from metastasis advantages various cancers.[32,33] In this study, we evidenced that DGT exerted NF-κB translocation by blocking TGF-β mediated ERK activation. Likewise, Auyeung *et al.*[34] reported that Astragalus saponins, promote apoptosis and inhibit cell growth in colon cancer HT-29 through ERK activation with continuous NF-κB/DNA binding activity and thus support DGT cancer inhibition properties.

In cancerous conditions, the noncanonical TGF-β pathway predominantly regulates ERK signalling cascade which leads to stimulates NF-κB, this causes evokes invasion and prohibition of apoptosis.[35] Hence, downregulation of TGF-β is linked to suppressing ERK/NF-κB responses and reversing tumour progression and improving survival rates. Our results demonstrated that interruption of TGF-β signals by DGT prevents ERK/NF-κB nuclear translocation and activates JNK, p38 which ensues invasion factors inhibitions and simultaneously induces apoptosis. Furthermore, DGT docking results indicate DGT binds to TGF-β, and ERK active sites suggest target interaction and inhibition of TGF-β by DGT. Similarly, steroid saponin ginsenosidess suppresses TGF-β induced tumour growth, and invasion via suppressing the NF-κB/ERK pathways.[36] Besides, dioscin a saponin from *Solanum nigrum* inhibits TGF-β signals to suppress A549 lung cancer migrations and invasion.[37,38] Therefore, these findings strongly proposed that DGT inhibits carcinogenesis and induces apoptosis through the target TGF-β/ERK/NF-κB signalling pathway.

CONCLUSION

Overall, our results indicated that DGT subdues invasion, and induced apoptosis in human oral cancer cells through interferer pathways involving TGF-β/ERK/NF-κB signalling, thus also stimulating Bax-induced apoptosis. Therefore, these results strongly suggested that inhibition of non-canonical TGF-β signalling is an important mechanism for induction of apoptosis and DGT has a potent novel candidate against OSCC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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