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Original Article

10-Gingerol as an inducer of apoptosis through HTR1A in cumulus cells: *In-vitro* and *in-silico* studies

Kiptiyah Kiptiyah, D.Sc [a,*], Widodo Widodo, Ph.D [b], Gatot Ciptadi, D.Sc [e], Aulanni'am Aulanni'am, DVM [d], Mohammad A. Widodo, Ph.D [c] and Sutiman B. Sumitro, D.Sc [b]

[a] *Department of Biology, Faculty of Sciences and Technology, Maulana Malik Ibrahim Islamic State University of Malang, Indonesia*
[b] *Department of Biology, Faculty of Sciences, Brawijaya University, Malang, Indonesia*
[c] *Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[d] *Department of Chemistry, Faculty of Sciences, Brawijaya University, Malang, Indonesia*
[e] *Department of Reproduction, Faculty of Husbandry, Brawijaya University, Malang, Indonesia*

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الملخص

أهداف البحث: تلعب الخلايا المحيطة بالبويضة دورا حاسما كوسيط أساسي في نضج البويضات. ويحتوي الزنجبيل على ١٠-جينجيرول، الذي يمكن أن يحفز موت الخلايا المبرمج في خلايا سرطان القولون. واستنادا إلى هذه الفرضية، هدفت هذه الدراسة إلى تحديد ما إذا كان ١٠ جينجيرول قادرا على تحريض موت الخلايا المبرمج في خلايا طبيعية، وتحديدا الخلايا المحيطة بالبويضة.

طرق البحث: استخدمت هذه الدراسة التحليل المختبري للخلايا المحيطة بالبويضة المستزرعة في محلول م ١٩٩ والمحتوي على١٠ جينجيرول في تركيزات مختلفة (١٢، ١٦، و٢٠ ميكرومول) ومن ثم الكشف عن نشاط قاتل للخلايا مبكر باستخدام عدة أنيكسين - فلورسين أيسو ثايو سايانيت للكشف.

النتائج: تُظهر البيانات في التجارب المختبرية أن عدد خلايا الموت المبرمج زادت مع زيادة فترة الحضانة: ١٢ ميكرومول (٦٣.٧١٪ ± ٢.١٩٢٪)، ١٦ ميكرومول (٧٤.٥١٪ ± ٤.٥٩٦٪)، و ٢٠ ميكرومول (٧٨.٧٩٥٪ ± ١.٤٣٥٪). تستطيع المادة ١٠ جينجيرول أن تحفز الموت المبرمج للخلايا في الخلايا المحيطة بالبويضة، إلا أنها تثبط وظائف الجين المسؤول عن برمجة مستَقبِل 5- هيدروكسي تريبتامين وتبطل عمل كلا من جلايكوجين سنثايز كاينايز ٣ بيتا والجين المبرمج لـ " أي كي تي - ١ ".

الاستنتاجات: تشير نتائج هذه الدراسة إلى أن هناك حاجة لمزيد من الفحص لـ ١٠-جينجيرول كعامل منع للحمل.

الكلمات المفتاحية: ١٠ جينجيرول؛ الموت المبرمج للخلايا؛ الجين المسؤول عن برمجة مستَقبِل 5- هيدروكسي تريبتامين؛ الخلايا المحيطة بالبويضة؛ في المختبر؛ في السيليكو

* Corresponding address: Jl. Gajayana No. 50, Malang 65144, East Java, Indonesia.
E-mail: qibthiyah@yahoo.com (K. Kiptiyah)

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Abstract

Objectives: Cumulus cells play a crucial role as essential mediators in the maturation of ova. Ginger contains 10-gingerol, which induces apoptosis in colon cancer cells. Based on this hypothesis, this study aimed to determine whether 10-gingerol is able to induce apoptosis in normal cells, namely, cumulus cells.

Methods: This study used an *in vitro* analysis by culturing Cumulus cells in M199 containing 10-gingerol in various concentrations (12, 16, and 20 μM) and later detected early apoptotic activity using an Annexin V-FITC detection kit.

Result: The *in vitro* data revealed that the number of apoptosis cells increased along with the period of incubation as follows: 12 μM (63.71% ± 2.192%); 16 μM (74.51% ± 4.596%); and 20 μM (78.795% ± 1.435%). The substance 10-gingerol induces apoptosis in cumulus cells by inhibiting HTR1A functions and inactivating GSK3B and AKT-1.

Conclusions: These findings indicate that further examination is warranted for 10-gingerol as a contraception agent.

Keywords: 10-Gingerol; Apoptosis; Cumulus cells; HTR1A; *In silico*; *In vitro*

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Introduction

Cumulus cells are a group of granulosa cells that surround oocytes and support the maturation process.[1] Cumulus cells play a crucial role in modulating signals,[2] nourishing bioactive nutrients,[3,4] and transporting them through gap junctions for oocyte maturation stimuli.[5] The role of the cumulus cell is an essential mediator for ovulation stimuli.[6] Prevention of ovulation is a major target mechanism of oral contraceptives.[7] The use of these contraceptives often results in side effects for their acceptor, from general conditions, such as obesity,[8] to serious cases, such as breast, cervical, endometrial, or colorectal cancer.[9] Therefore, we need to uncover an agent, such as ginger, which contains 10-gingerol, as a candidate contraception agent.

Since ancient times, ginger has been used as a traditional drug without side effects.[10,11] The herbal drug ginger (*Zingiber officinale* Roscoe) is rich in various pharmacological properties, including antioxidant,[12] anti-inflammatory,[13] and anti-cancer[11,14] activities through the activation of p-53 to induce apoptosis.[15,16] Bioactive compounds of ginger induce apoptosis to suppress ovarian cancer cell proliferation.[17] Terpenoid of ginger alters the Bax/Bcl-2 ratio to induce apoptosis in MDA-MB-231 human breast cancer cells[18] followed by a down regulation of cellular pro-caspase 3.[19] A minor bioactive component in fresh ginger is shogaols,[20] which induces apoptosis through the up-regulation of p-53 and the down-regulation of B cell lymphoma-2 (Bcl-2) followed by cytochrome Complex (cyt c) release, perturbation of apoptosis proteins and activation of caspase 9 and 3.[21] Fresh ginger also contains gingerols, such as 6-gingerol, 8-gingerol, and 10-gingerol, which are major biologically active components.[22] 6-Gingerol has various pharmacological functions and exhibits anti-proliferative and pro-apoptotic activities.[23] In MDA-MB-231 breast cancer cells, 8-gingerol and 10-gingerol are pro-apoptotic and anti-proliferative.[24] In HL-60 human leukaemia cells, 10-gingerol is anti-carcinogenic and kills cells at concentrations of 10−100 μM, which is better than 6-gingerol.[25] In HCT 116 and HT 29 colon cancer cell lines, the bioactive molecules in ginger act as anti-tumour agents by inducing apoptosis.[26] In HCT 116 colon cancer cells, 10-gingerol inhibits cell proliferation through the induction of mitochondrial apoptosis.[27] Recently, a study reported that 10-gingerol was more potent than 6-gingerol and 8-gingerol for the inhibition of mammary carcinoma cell growth through the induction of apoptosis.[28]

The bioactive compound in ginger can interact with a receptor of serotonin, HTR1A.[29] HTR1A has anti-apoptotic properties[30] and is implicated in cell survival.[31] HTR1A is found in cumulus cells and plays a crucial role in the regulation of cell proliferation,[32] which is inhibited by an antagonist of HTR1A.[33] HTR1A functions are disturbed by a ligand inhibitor, H1C1.[34] Gingerols are major compounds in ginger, which exhibit anti-cancer effects through degradation of the GSK3B pathway.[35] The GSK3B has positive functions, which are related to the regulation of numerous cellular functions, including cell growth[36] and cell cycle,[37] whereas the negative functions of GSK3B are related to the regulation of the expression of p-53 to induce apoptosis.[38] 10-Gingerol inactivates AKT to regulate down regulate the cell cycle process in MDA-MB-231 breast cancer cells and inhibit their proliferation.[39]

The AKT inactivation is associated with cell death.[40] AKT is a serine/threonine-specific protein kinase that is a mediator through the PI3K/AKT pathway in biological processes, such as cell proliferation, survival cell,[41] cell cycle,[42] and apoptosis.[43] Apoptosis is the process of programmed cell death, which can occur either through the extrinsic pathway, which is characterized as death receptor-mediated,[44] and the intrinsic pathway, which is influenced by members of the bcl family (bax and bcl-2) to act as pro- or anti-apoptotic factors depend on the regulatory proteins.[45] The activation of pro-apoptotic proteins, such as FOXO-1[46], FOXO-3,[47] and PTEN,[48] and the inactivation of anti-apoptotic proteins, such as ILK,[49] MDM2,[50] NOS3,[51] mTOR,[52] and RICTOR,[53,54] leads to apoptosis.

During apoptosis, phospholipid phosphatidylserine residues are translocated from the inner to the outer leaflet of the plasma membrane.[55] In this case, phosphatidylserine binds to the protein Annexin-V when it is labelled with a fluorescent FITC tag, which detects apoptosis.[56] Increased apoptosis in cumulus cells is an indicator that decreases the quality of the oocyte, which contributes to a decrease in the percentage of oocyte maturation.[57] The oocytes maturation rate is higher in oocytes with the cumulus cells than in oocytes without the cumulus cells.[58] The relationship between the cumulus cells and the oocyte is important, not only in oocyte maturation but also for secreting chemotactic factors that guide the spermatozoon to the oocyte, which increases the chance of fertilization.[59]

To gain information about its side effects when it is applied for cancer treatment, we attempted to investigate the effects of 10-gingerol, a bioactive compound of ginger on cumulus cells. While the anti-carcinogenic property of 10-gingerol has been reported previously, this study is the first to investigate the effects of 10-gingerol on normal cumulus cells.

Materials and Methods

These studies obtained ethical approval from the Health Research Ethics Committee at the Medical Faculty of Brawijaya University in Malang, East Java, Indonesia No. 588/EC/KEPK/11/2014.

Isolation of cumulus cells

The ovaries of a goat were obtained from an abattoir and were saved in 0.9% NaCl at 34−37 °C. The cumulus cells were released from 3 to 5 mm ovarian follicles using a 21 Gx1.5 in needle, were diluted with 1 mL of M199 (Sigma) and were centrifuged for 5 min. The supernatant was put aside, and then, 1 mL of fresh M199 (Sigma) was added.

Cumulus cell culture and harvesting

The cumulus cells were cultured using M199 in four composition mediums consisting of 20 mM high D-glucose (Merck) as a control and three combinations (i.e., the high glucose medium contained 10-gingerol (Aphios) in various

concentrations (12, 16, and 20 μM)) as treatments. The cells were incubated for 24, 48, 72, and 96 h in a humidified atmosphere of 5% CO_2 at 37 °C. The cumulus cells were harvested using trypsin−EDTA (Sigma).

Detection of apoptosis in cumulus cells

The cumulus cells were harvested, washed using cold Biolegend cell staining buffer, and resuspended in Annexin V binding buffer, and 100 μL of the cell suspension was transferred and added to 5 μL of fluorochromes into the conjugated Annexin V-FITC. Then, 10 μL of the PI solution was added, and it was incubated for 15 min at 25 °C in the dark room. Next, we added 400 μL of the Annexin V binding buffer and analysed the samples using Flow Cytometry. The data are presented as dot plots (Annexin V-FITC plotted against PI staining). Apoptosis was determined based on the Annexin V-FITC positive cumulus cells (i.e., UR (upright) + LR (low right)).

Prediction target and pathway analysis of 10-gingerol using a bioinformatics approach

The protein targets of 10-gingerol were assessed using SwissTarget (http://swisstargetprediction.ch). We found HTR1A as a protein target of the compound (at 90% probability). The three-dimensional structure (3D) of HTR1A was modelled using homology modelling (SWISS-MODEL) based on a protein template (PDB 2JOA). The 3D model was used to examine the binding between HTR1A and 10-gingerol. The interaction between the both molecules was analysed using AutoDock Vina in the PyRx 0.8 Program. The results of the docking were visualized using BIOVIA 1 Discovery Studio 2016'-Accelrys (San Diego, CA, USA). Furthermore, we explored the function of HTR1A through the STRING database (http://string-db.org/newstring).

Statistical analysis

This experiment consisted of 16 combination treatments, and all treatments were performed at least two times. The statistical significance of apoptosis in the cumulus cells was determined using a two-way analysis of variance (ANOVA) test using the SPSS 16 programme. The results were presented as the mean ± standard deviation (SD) in the histogram.

Results

The result of the *in vitro* study showed that apoptosis in the cumulus cells was minimal and was statistically significant only at the end of the 96 h incubation period (Figure 1A4, Table 1, Figure 2). The apoptosis percentage in the cumulus cells increased with the 10-gingerol treatment along with the period of incubation. In this case, this was proven by the treatments that were described in the data in Figure 1D1−D4, Table 1, and Figure 2. The data revealed that the high glucose medium containing 20 μM of 10-gingerol increased the apoptosis percentage of the cumulus cells for all the tested incubation periods as follows: 24 h ($16.07\% \pm 0.36\%^{j}$) < 48 h ($32.01\% \pm 2.33\%^{g}$) < 72 h ($53.69\% \pm 2.40\%^{d}$) < 96 h ($78.8\% \pm 1.44\%^{a}$) as well as in the highest incubation period (96 h), which is presented in the data in Figure 1B4, C4, D4, Table 1, and Figure 2 and revealed that 12 μM ($63.71\% \pm 2.19\%^{c}$) < 16 μM ($74.51\% \pm 4.6\%^{b}$) < 20 μM ($78.8\% \pm 1.44\%^{a}$).

The results of the analysis using the Discovery Studio Visualizer revealed that 10-gingerol (Figure 3A) binds to its protein target HTR1A, which binds to ILE 385, GLY 384, ILE 383, and ARG 386 by hydrogenic binding and forms alkyl bonds to ILE 415 and ARG 386. In addition, 10-gingerol also forms hydrophobic bond and binds to TYR 382, ARG 386, ILE 418, GLU 416, LYS 381, ILE 452, and MET 387 (Figure 3B) with a hydrophobicity between −3 and 3 (Figure 3C), which indicates that 10-gingerol binds to hydrophobic active sites.

The result of the exploration using the STRING database (http://string-db.org/newstring) suggested that HTR1A functions are linked to various biological process pathways, including regulation of the apoptotic process, the cell cycle process, and the regulation of cell proliferation. The presence of 10-gingerol inhibits HTR1A functions, inactivate GSK3B and AKT-1 to induce apoptosis, and inhibits the cell cycle process and the proliferation of cumulus cells by activating pro-apoptotic proteins, such as FOXO1, FOXO3, and PTEN and inactivating anti-apoptotic proteins, such as NOS3, ILK, MDM2, mTOR, and RICTOR (Figure 4).

Discussion

Details of apoptosis in cumulus cells have been investigated. However, the role of 10-gingerol to induce apoptosis that involves its protein target HTR1A remains unclear. Glucose represents a critical physiological function that provides energy to support cumulus cell proliferation (Figure 1A1−A4, Table 1, Figure 2). Previous researchers have reported that high glucose concentrations promote the proliferation of cells and significantly increase the population of the cells.[60] Higher glucose concentrations produce higher cell populations than lower glucose concentrations.[61] A high glucose concentration (10−50 mM) promotes cell proliferation without any effect on viability.[62] The *in-silico* study predicted that apoptosis in the cumulus cells, by 10-gingerol, occurred through diffusion on the surface of the cumulus cell membrane and inhibited its protein target or receptor, HTR1A. Furthermore, 10-gingerol crossed the cumulus cell's membrane and damaged the mitochondrial membrane, releasing cytochrome C from the mitochondria to go into the cytosol. Ziegler & Groscurth[63] reported that, in the cytosol, cytochrome C activated pro-caspase 9, 3, 6, and 7. These caspases led to apoptosis in the cumulus cells.[64] In addition, a previous study reported that 10-gingerol is a hydrophobic compound[19] and possesses anti-carcinogenic properties that are better than other gingerols.[25] 10-gingerol, a lipophilic compound, inhibits the proliferation of cancer cells and decreases the viability of tumour cell lines,[24] and 10-gingerol induces apoptosis in a cancer cell.[27]

10-gingerol is a hydrophobic compound that acts as an inhibitor for HTR1A functions (Figure 3) to induce apoptosis (Figure 4). Previous researchers report that ginger possesses phenolic compounds that can interact with HTR1A.[29] HTR1A stimulates numerous biological processes, such as cell proliferation,[65] growth regulation,[33,66] anti-apoptosis,[30]

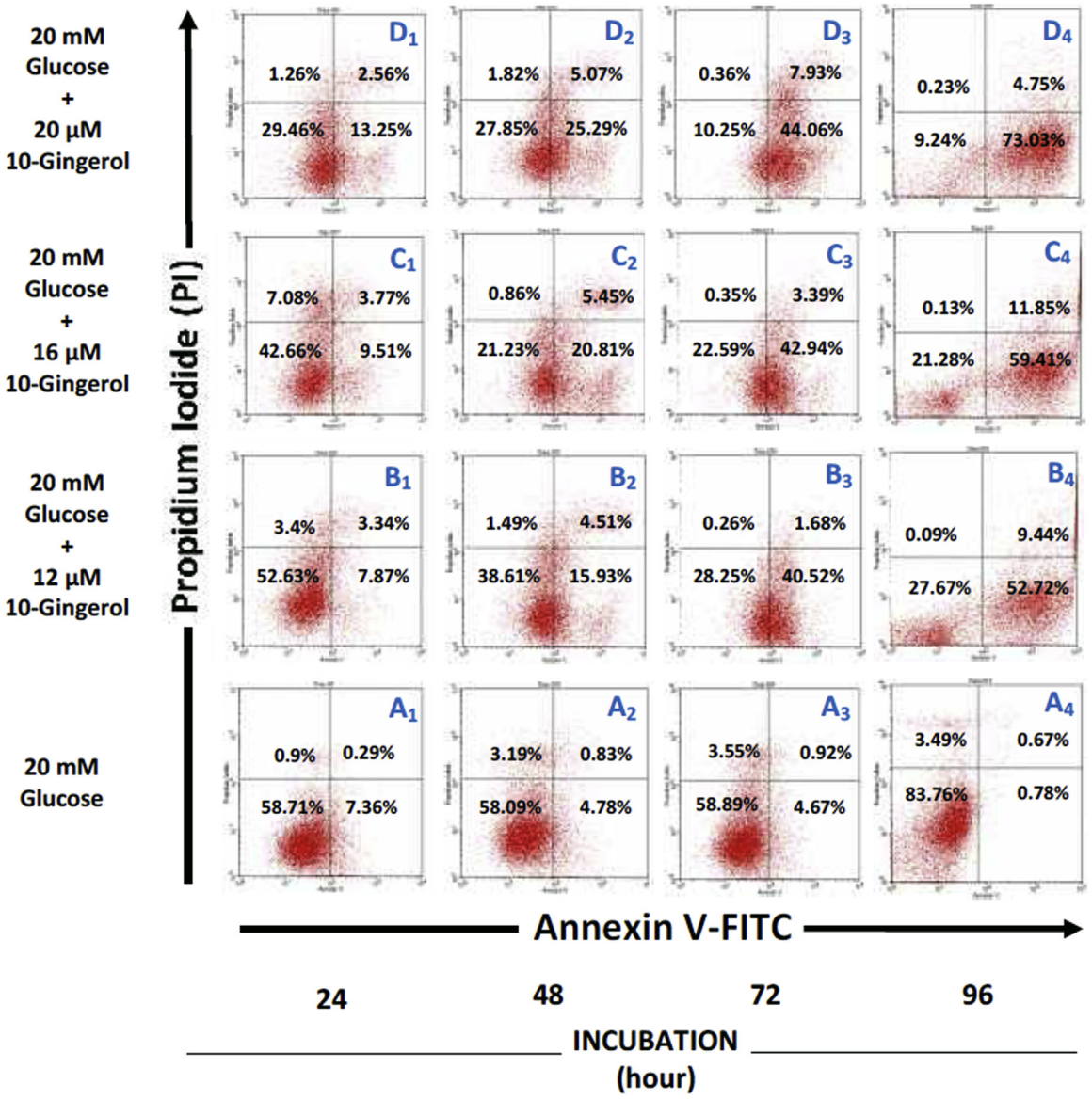

Figure 1: Percentage of apoptotic cumulus cells resulting from the 10-gingerol treatment for an incubation period of 24–96 h. The cumulus cells were cultured using 20 mM high glucose medium and high glucose medium contained various concentrations of 10-gingerol for an incubation period of 24–96 h. The induction of apoptotic cumulus cells was investigated using an Annexin V-FITC apoptosis detection kit with a PI solution and was analysed by flow cytometry. The data were presented as dot plots (Annexin V-FITC plotted against PI staining). Apoptosis was determined based on the Annexin V-FITC positive cumulus cells, i.e., UR (Up-Right) + LR (Low-Right).

Table 1: Two-way ANOVA of the apoptosis percentage in the cumulus cells resulting from the 10-gingerol treatment over incubation periods of 24, 48, 72, and 96 h.

Treatments	Apoptosis percentage in the cumulus cells			
	24 h (Mean ± SD)	48 h (Mean ± SD)	72 h (Mean ± SD)	96 h (Mean ± SD)
20 mM glucose	8.60 ± 1.34^{l}	5.64 ± 0.04^{m}	5.04 ± 0.79^{m}	1.36 ± 0.13^{n}
20 mM glucose + 12 µM 10-gingerol	10.05 ± 1.64^{kl}	21.78 ± 1.89^{i}	44.26 ± 2.91^{f}	63.71 ± 2.19^{c}
20 mM glucose + 16 µM 10-gingerol	12.77 ± 0.72^{k}	27.08 ± 1.15^{h}	49.14 ± 3.97^{e}	74.51 ± 4.60^{b}
20 mM glucose + 20 µM 10-gingerol	16.07 ± 0.36^{j}	32.01 ± 2.33^{g}	53.69 ± 2.40^{d}	78.80 ± 1.44^{a}

Mean ± SD with different letter means that there is a significant difference ($p < 0.05$), and if the letter are the same, there is not a significant difference ($p > 0.05$).

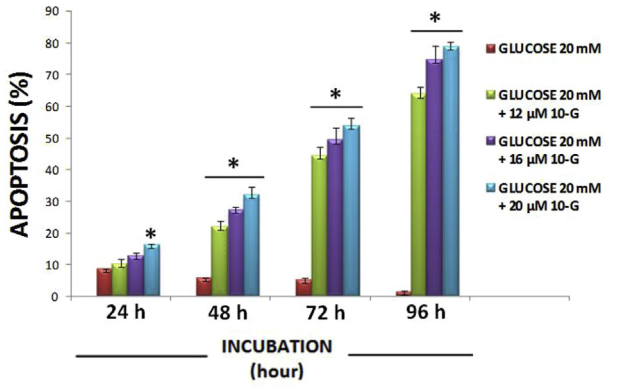

Figure 2: The cumulus cells were cultured in 20 mM high glucose medium and high glucose medium containing various concentrations (12, 16, 20 µM) of 10-gingerol (10-G) over an incubation period of 24–96 h. Apoptotic cumulus cells were measured by flow cytometry. *$p < 0.05$ was significantly different from the control. The data are presented as the Mean ± SD.

NF-κB pathway,[67] modulating cell survival,[31] controlling cell development,[68] cell survival,[67] and cell cycle progression.[69] In contrast, the inactivation of HTR1A, by an inhibitor, disturbs the cell cycle process.[70] The HTR1A that was applied in the *in-silico* study was for the anti-proliferation of ligands.[34] In fact, HTR1A was also found in cumulus cells.[32] The HTR1A acts as a pro-apoptosis agent[43] through the negative role of GSK3B for p-53 expression regulation,[36] which induces apoptosis[38] through AKT inactivation by 10-gingerol[27] and induces G1 phase arrest.[39]

The AKT inactivation is associated with cell death[40] and decreases cell proliferation.[71,72] The inhibition of the AKT pathway acts to enhance the activation of pro-apoptosis proteins, such as FOXOs[73] as tumour suppressors,[74] and their forced expression inhibits cell proliferation.[75] FOXOs (FOXO1 and FOXO3) are important targets of AKT (Figure 4) that play a role in promoting cell growth inhibition and apoptosis by inducing the expression of multiple pro-apoptotic proteins, such as the Bcl2, Fas ligand, and tumour necrosis factor-related apoptosis-inducing ligand, TRAIL.[76] FOXO1 acts as a tumour suppressor,[77] an anti-proliferative, and a pro-apoptotic gene that might lead to endothelial cell death.[78] The activation of FOXO1 is involved in the regulation of apoptosis, cell proliferation arrest, and decreases cell viability in cervical cancer cell lines and during tumourigenesis.[46] In addition, it acts as a tumour suppressor[79] and function in the process of pro-apoptosis in the follicular granulosa cells of growing follicles.[62,80] The expression of FOXO1 induces pro-apoptotic pathways and delays the G2/M transition.[81] FOXO3 is a pro-apoptotic molecule that increases in follicular atresia.[47] The inactivation of AKT increases FOXO3a, which is a subfamily of FOXO3 that functions to suppress tumour cell growth.[82]

AKT inhibition suppresses cumulus cell expansion,[83] and in pancreatic islet β-cells, it results in a dysfunction that leads to apoptosis through PTEN activation.[84] PTEN is a tumour suppressor that induces apoptosis by activating caspase-3 and caspase-8,[48] inhibiting cell proliferation,[85] and increasing apoptosis.[86] The activation of PTEN activates p53 and their interactions induce apoptosis through the Bax and caspase 3 pathways.[87] PTEN and p53 also form a complex in the nucleus and induce the expression of tumour suppressors, resulting in apoptosis[88] and the induction of cell cycle arrest at the G1 phase to inhibit cell proliferation.[89]

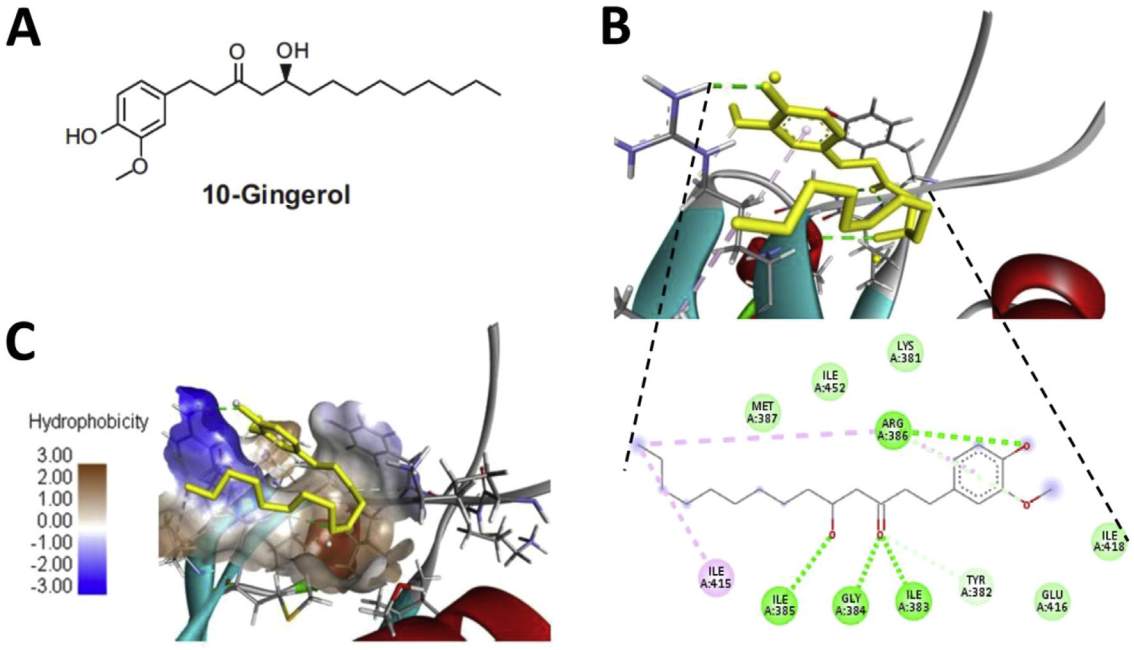

Figure 3: Result of the visualization using BIOVIA Accelrys 2016 exhibits an interaction between 10-gingerol and HTR1A. The chemical structure of 10-gingerol (Qiu et al., 2015) as a ligand (A). The 10-gingerol (yellow) binds to HTR1A (grey) by hydrogenic, alkyl, and hydrophobic binding that involves some amino acids (B) in hydrophobicity −3 until 3 (C).

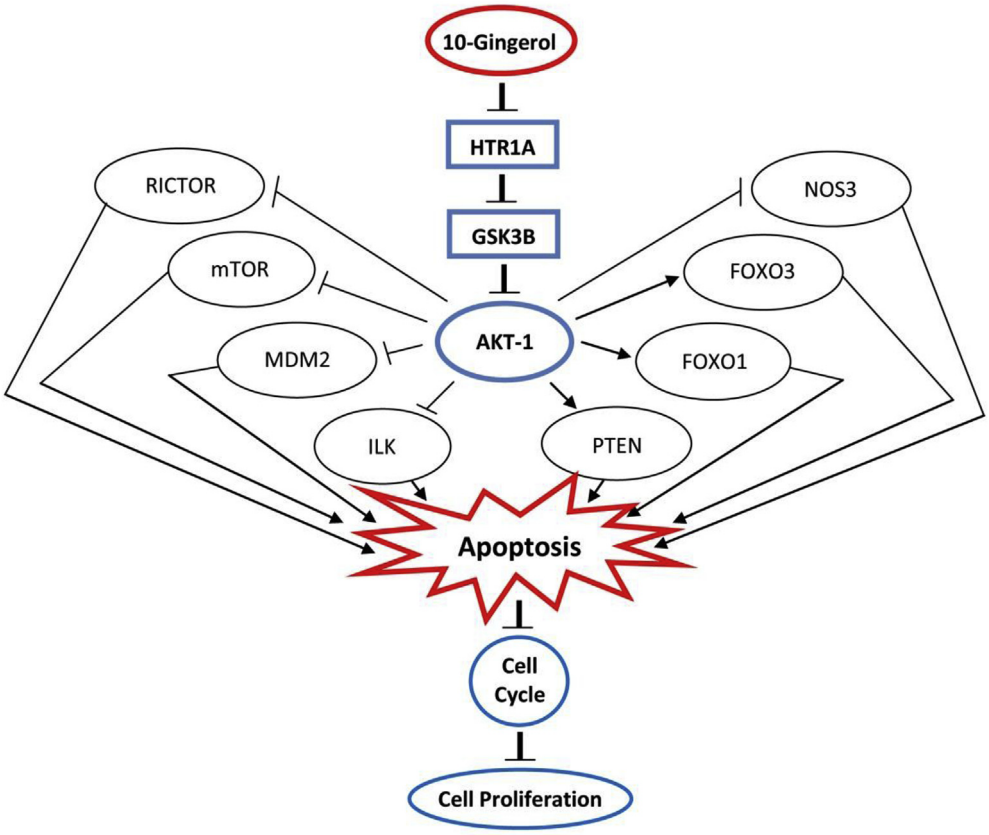

Figure 4: Result of the exploration of HTR1A functions involved in biological process pathways using the STRING database (http://string-db.org/newstring). The pathway analysis exhibited the regulation of apoptosis, cell cycle process, and proliferation by 10-gingerol through HTR1A in the cumulus cells.

The inactivation of AKT inactivates anti-apoptosis proteins, such as NOS3, an enzyme that plays a crucial role in the production of NO.[90] NO is indicated to protect against the effects of free radicals, DNA damage, and impair the tumour suppressor function of p53, which may cause cancer cell development.[91] NO directly inhibits the activity of caspases to obstruct apoptosis by blocking cytochrome C release, increasing Bcl-2 expression, which controls mitochondrial permeability, and playing a role in tumour progression.[92] The absence of AKT-1 enhances apoptosis as a result of the loss of eNOS, which is well known as NOS3.[93] Lower levels of NOS3 result in lower levels of NO, which are associated with poorer cell survival.[94] An altered intracellular generation of NO from NOS-3 induces cell death and arrests cell proliferation in tumour cells.[95]

AKT inactivation suppresses ILK expression.[96] ILK is a serine−threonine kinase that is involved in the regulation of cell proliferation.[97] The knockdown of ILK expression induces growth inhibition and apoptosis in ovarian cancer cells.[98] The inhibition of ILK *in vivo* results in decreased tumour growth through the induction of apoptosis,[49] which induces cell-cycle arrest[99] and proliferative defects.[100] Our *in-silico* data provides evidence that 10-gingerol is a small molecule inhibitor for the expression of ILK that induces apoptosis in cumulus cells (Figure 4).

Increasing apoptosis by 10-gingerol (Table 1, Figure 2) through HTR1A induced the inactivation of AKT and mTOR (Figure 4). The inactivation of both AKT activity and mTOR enhances the apoptotic cell death of granulosa cells,[51] because mTOR is a serine/threonine kinase[101] that plays a critical role as a growth factor in mammals.[102] mTOR is a major downstream target of AKT[52] that form two functionally distinct complexes, which involve mTORC1 and mTORC2.[103,104] mTORC1 is essential to control protein synthesis and cellular metabolism, while mTORC2 is essential to control cell viability.[105] AKT inhibition, followed by mTORC1 blockage, contributes to anti-tumour responses.[106] The down-regulation of mTORC2 facilitates apoptosis in breast cancer cells.[105] The suppression of mTOR blocks G1 cell cycle progression[107] and inhibits the proliferation of a lymphoma cell line.[108] In addition, the inhibition of the AKT-mTOR pathway leads to the initiation of apoptosis[52] and cell cycle G1 arrest in ovarian cancer cells,[109]which accompanies cell death,[110] resulting in anti-tumour effects.[111]

Apoptosis in cumulus cells is significantly increased by 10-gingerol (Table 1, Figure 2). In this case, 10-gingerol inhibits HTR1A functions (Figure 3) to suppress RICTOR (Figure 4). RICTOR is a cytosolic protein that is recognized as a specific component of mTORC2, which functions to regulate cell proliferation.[101] The loss of RICTOR prevents mTORC2 activation.[112] In addition, the inhibition of RICTOR induces apoptosis to inhibit cell proliferation.[113] Moreover, the loss of RICTOR in oocytes causes follicular apoptotic death,[54] while the deletion

of RICTOR in osteoblasts inhibits osteoblast bone formation.[114] Furthermore, the suppression of RICTOR induces apoptosis in lung cancer cells.[53]

Apoptosis in the cumulus cells by 10-gingerol (Figure 1), through HTR1A, occurred by inhibiting MDM2 activity, which involved the blockade of AKT (Figure 4). The inhibition of AKT re-activates the promotion of p-53 function to block MDM2 activity,[115–117] which, thus, induces the apoptotic response and G2/M cell cycle arrest.[118] The inhibition of MDM2 results in the activation of p53 and the induction apoptosis through the activation of caspases 3, 7 and 9.[50] The disruption of the p-53-MDM2 interaction activates the p-53 pathway, resulting in apoptosis,[119] which is cytotoxic to the G1 phase arrest and the anti-proliferative effect.[120] The inactivation of AKT contributes to MDM2 depletion, which, thus, the results in increased apoptosis, cell cycle arrest, and anti-proliferative effects.[121]

Conclusion

This study's results indicate that 10-gingerol induces apoptosis and inhibits the cell cycle process and cell proliferation in cumulus cells through HTR1A, which inactivates the GSK3B protein and the AKT-1 protein. Consequently, this protein activates pro-apoptosis proteins, such as FOXO-1, FOXO-3, and PTEN, and inactivates anti-apoptosis proteins, such as ILK, MDM2, mTOR, NOS3, and RICTOR. Furthermore, ginger extract with 10-gingerol can be further utilized as an alternative contraceptive agent as it prevents oocyte maturation through the cumulus cells death.

Abbreviations: ARG, arginine; eNOS, endothelial nitric oxide synthase; FOXO, forkhead box; GLU, glutamine; GLY, glycine; GSK3B, glycogen synthase kinase-3β; HTR1A, 5-hydroxytryptamine receptor 1 A; ILE, ileusine; ILK, integrin-linked kinase; LYS, lysine; MDM2, murine double minute clone 2; MET, methionine; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; NO, nitric oxide; NOS3, nitric oxide synthase 3; PTEN, phosphatase and tensin homologue delete on chromosome ten; RICTOR, rapamycin-insensitive companion of mTOR; TYR, tyrosine

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

KK conceived, designed, and performed the experiment, provided research materials, collected, organised, analysed, and interpreted the data, wrote the paper and revised it to be published. WW conceived, designed, analysed, and interpreted the *in-silico* data, provided the *in silico* journal research, reviewed drafts of the paper to be submitted, and give the final approval of the version to be published. GC conceived and designed the *in vitro* experiment, provided information about the research materials, reviewed the drafts of the paper to be submitted, and gave the final approval of the version to be published. AA conceived and designed the *in vitro* study, reviewed the drafts of paper to be submitted, and gave the final approval of the version to be published. MAW conceived and designed the *in vitro* study, helped in data interpretation, reviewed the drafts of the paper, and gave the final approval of the version to be published. SBS conceived and designed the study, supervised the development of the work, helped in data interpretation, reviewed drafts of the paper, and gave the final approval of the version to be published.

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