Synergistic Genotoxic Effects and Modulation of Cell Cycle by Ginger Ethanolic Extracts in Adjunct to Doxorubicin in Human Lymphocytes In Vitro

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Background: *Several natural phytochemicals are increasingly used, as an adjunct to chemotherapy, to reduce drug adverse effects. Zingiber officinale rhizome (ginger) product has been reported to be effective against nausea and vomiting in patients receiving emetogenic chemotherapy such as cisplatin/doxorubicin (DXR). In addition, its ethanolic extract of Zingiber officinale rhizome (EEZOR) has been reported to possess anticarcinogenic properties. However, the mechanism for anticancer activity of ginger especially when used in combination with chemotherapy has not well elucidated, one of its possible mechanisms might involve its genotoxicity.*

Objective: *To investigate genotoxic and cytotoxic potentials of EEZOR alone and EEZOR pretreatments followed by 0.1 mcg/ml DXR, a genotoxic chemotherapeutic agent in human lymphocytes by sister chromatid exchange (SCE) assay in vitro. The effect on cell cycle kinetics was also explored.*

Material and Method: *Human lymphocytes were treated with EEZOR alone at 25-500 mcg/ml and EEZOR pretreated at 12.5-200 mcg/ml followed by 0.1 mcg/ml DXR. SCE levels and cell cycle kinetics were evaluated.*

Results: *EEZOR significantly induced biphasic SCE at 50 and 400 mcg/ml ($p<0.05$). However, cytotoxicity manifested at 500 mcg/ml. All EEZOR pretreatments at 12.5, 25, 50, and 100 mcg/ml, except at 200 mcg/ml, prior to DXR, moderately enhanced DXR-induced genotoxicity by 1.3 times ($p<0.05$). Both EEZOR alone and EEZOR prior to DXR at certain concentrations delayed cell cycle.*

Conclusion: *At specific doses, EEZOR could induce genotoxicity and in pretreatments could moderately enhance DXR-induced genotoxicity and delay cell cycle. This finding suggests that dosage use of EEZOR needs to be adjusted for long-term safety. In addition, EEZOR in adjunct to DXR might have potential benefits not only as an emetic agent but also in chemotherapy. Further in vivo animal and human studies to support this evidence is essentially needed.*

Keywords: *Cell cycle, Genotoxicity, Doxorubicin, Human lymphocyte, Sister chromatid exchange, Zingiber officinale rhizome*

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Cancer is a major cause of mortality and morbidity in human. Most of the present chemotherapeutic compounds for cancer have limitations to their efficacy and also have possible adverse side effects. For example, doxorubicin (DXR), an anthracycline antibiotic used to treat cancer, such as breast and ovary cancers, can cause serious heart problems and leukemia[1]. Cisplatin (cis-diamminedichloroplatinum), an alkylating agent for cancers of the bladder, ovary and lung, can induce nephrotoxicity[2]. Combining proven traditional herbal medicine with these chemotherapeutic agents might reduce those adverse effects as well as increase the efficacy of chemotherapy.

Zingiber officinale Roscoe (family Zingiberaceae), commonly known as ginger, or "Khing"

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in Thai, a perennial rhizomatous herb, is used as a spice and for traditional medicine, especially in Asia and Africa. It is used to balance "Yin-Yang" in the human body, especially against weakness or imbalance in circulation. The ethanolic extract of *Zingiber officinale* rhizome (EEZOR) has been demonstrated to have various properties such as antiemetic[3], anti-inflammatory[4,5], antimicrobial[6], antithrombotic[7], antitumor[8], and gastroprotective activities[9]. Ginger reduces acute chemotherapy-induced nausea in adult patients[10] and in children and young adults with sarcoma who received cisplain/doxorubicin chemotherapy[11], but not in an acute phase of cisplatin-induced emesis in gynecological cancer[3].

As well as its individual use, ginger is used as a primary ingredient in varying recipes of Thai traditional medicine. For example, one-third volume of EEZOR is contained in "Prasakanplu" (formulation for anti-dyspepsia) and in "Pikuttreeratagula" (formulation for anti-flatulant). One-fifth volume of EEZOR is contained in "Pikutbenjakul" (formulation for balancing the body before chemotherapy). However, the mechanism underlying the actions of ginger has not yet been well elucidated.

There are a few reports about cytotoxic potentials of EEZOR in human cancer cell lines. Two studies demonstrated that the EEZOR induced cytotoxicity against large lung carcinoma cell line (COR-L23)[12] and cholangiocarcinoma[13]. Our previous study showed that the ethanolic extract of Pikutbenjakul significantly induced genotoxic and cytotoxic activities in human lymphocytes, and these effects might be due to its components such as EEZOR[14]. However, genotoxic and cytotoxic potentials of EEZOR in human lymphocytes has not yet been evaluated.

Certain bioactive phenolic compounds in EEZOR such as gingerols, paradols and shogaols exhibited anticancer, anti-inflammation and antioxidant activities[15]. Shogaols and gingerols inhibited growth of human lung cancer and colon cancer cells[16]. 6-Gingerol protected against DXR-induced cardiotoxicity[17] and prevent cisplain-induced acute renal failure in rats[18]. Yang et al (2010) showed that 6-gingerol also induced genotoxic effects in human hepatoma G2 cells[19].

In the current study, it is hypothesized that as EEZOR comprises various natural bioactive compounds, it might enhance chemotherapy. Therefore, a study was done to investigate the genotoxicity and cytotoxicity of EEZOR in human lymphocytes and its potential interference with cell cycle by sister chromatid exchange (SCE) in vitro. Modulation of these potentials by EEZOR in adjunct to DXR was also determined to study chemical biological interactions and effects.

Material and Method

Material collection

The rhizomes of *Zingiber officinale* Roscoe (family Zingiberaceae) were collected by folk healers from Khaokho, Petchabun, northern Thailand. The samples were then identified by the Herbarium of the Royal Forest Department of Thailand and designated as SKP1206261501.

Preparation of the EEZOR

Zingiber officinale rhizomes were cleaned with water, sliced and dried at 50°C. The dried materials were grounded to obtain a powder (10 mesh particle size) and then percolated with 95% ethanol for three days at room temperature. The product (EEZOR) was then filtered and dried with reduced pressure, and was kept at -20°C.

Chemical fingerprint study

Preparation of sample and standard compound

Prior to use, EEZOR was dissolved in acetronitrile, sonicated for 5 minutes and filtered through a 0.45 mm membrane filter. One milligram of 6-gingerol, purchased from Sigma-Aldrich, USA, was dissolved in 1 ml acetonitrile and used as a standard control.

Apparatus and chromatographic conditions

A chemical fingerprint of EEZOR was carried out to examine 6-gingerol by using a high performance liquid chromatography (HPLC) system (Constametric® 4100 Bio) as previously described by Plengsuriyakarn et al, 2012a. Briefly, a reversed-phase column, Phenomenex Luna 5 m C18 (2) 100A analytical column (250x4.60 mm 5 micron) was used. The mobile phase was composed of water to acetonitrile with gradient elution as follows: 0 min (55: 45), 8 min (50: 50), 17 min (35: 65), 32 min (0: 100), 38 min (0: 100), 43 min (55: 45), 48 min (55: 45); flow rate: 1 ml/min; UV detector: 282 nm; injection volume: 10 µl. Data were analyzed with TSP PC1000 software.

Sister chromatid exchange (SCE) assay

Cell cultures

Genotoxic study:

Lymphocyte-enriched buffy coat was cultured in a 5-ml culture medium using standard blood culture

conditions[20]. Approval for this experiment was obtained from the Institutional Ethics Committee (MTU-BC-3-CRO48-048/53). Twenty-four hours after the initiation of the culture, lymphocytes were centrifuged to obtain packed cells. The supernatant medium was removed and saved for reuse. The remaining lymphocytes were treated with EEZOR at concentrations of 25, 50, 100, 200, 400, and 500 µg/ml dissolved in 0.4% (V/V) DMSO in plain RPMI 1640 culture medium for three hours at 37°C. After treatments, all the lymphocyte cultures were centrifuged to pack the cells together and the supernatant was discarded. The treated lymphocytes were continued to be cultured at 37°C in the dark with the previously saved medium. Bromodeoxyuridine (BrdU) solution (Sigma-Aldrich, USA) was then added in the culture medium to give the final concentration of 5 µM. Doxorubicin (0.1 µg/ml) (Roche, Switzerland) was used as the positive control. Plain RPMI 1640 and 0.4% (V/V) dimethyl sulfoxide (DMSO) were used as the negative controls.

Drug interaction study:

Similar to the previous protocol lymphocytes were cultured using the same method. Cells were pretreated with EEZOR at concentrations of 12.5, 25, 50, 100, and 200 µg/ml (dissolved in 0.4% V/V DMSO) in culture medium for two hours at 37°C. Followed by centrifugation, the supernatant medium was discarded and the treated cells were continued to incubate with 0.1 µg/ml DXR for two hours at 37°C. For a positive control, the lymphocytes were simultaneously treated with plain RPMI 1640 culture medium or 0.4% V/V DMSO for two hours, washed and followed by DXR (0.1 µg/ml) for two hours. For negative control, treatment was made with plain RPMI 1640 for two hours followed by plain RPMI 1640 again for two hours. After treatments, all the lymphocyte cultures were centrifuged to pack cells together and the supernatant was discarded. The remaining cells were mixed with the previously saved medium and BrdU was added to a final concentration of 5 µM. The lymphocytes were then cultured at 37°C in the dark.

Cell culture harvest and staining

For genotoxic study of EEZOR the lymphocytes were harvested at 72 h after initiation whereas for drug interaction study of EEZOR/DXR the cells were harvested at 77 h after initiation because of cell cycle delay. All slides were prepared and stained using the fluorescent plus Giemsa technique, according to the standard protocol[21,22]. Twenty-five cells per dose in each experiment showing the second metaphase-staining pattern were scored from the coded slides for frequencies of sister chromatid exchange (SCE). The proliferation index (PI) and mitotic index (MI) were also evaluated to clarify cytotoxicity. The MI was determined as number of all mitotic cells/1,000 cells while the PI was determined as (MI+2MII+3MIII)/100 cells. MI is the number of metaphase cells from the first cell cycle (homogeneously-stained chromatids) (Fig. 1), MII is the number of metaphase cells from the second cell cycle (heterogeneously-stained chromatids) (Fig. 2), and MIII is the number of metaphase cells from the third cell cycle (mixed homogenously-stained and heterogeneously-stained chromatids) (Fig. 3). Two to three independent experiments were performed for each concentration of the treated compounds.

Statistical analysis

A square root transformation of the SCE data was required to stabilize the variances according to the procedure of Whorton et al 1984[23]. Frequency of transformed SCE was expressed as a square root of SCE. Dunnett's t-test was performed to analyze the difference between the means of the treated groups and the means of the control groups using the transformed data.

Transformed SCE = square root of SCE

Results

Chemical Fingerprint of EEZOR

The percentage yield of the EEZOR crude extracts of dried *Zingiber officinale* was 8.0 % w/w and

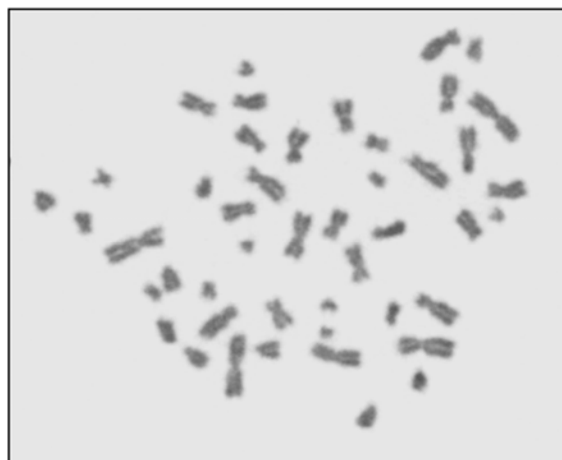

Fig. 1 Homogeneously stained chromatids in a metaphase cell from the first cell cycle (MI) in control group, plain RPMI.

6-gingerol concentration in EEZOR was 11.27±0.8 mg/g as analyzed by HPLC.

Genotoxic activities of the EEZOR

The data in Fig. 4 demonstrate that EEZOR only at concentrations of 50 and 400 μg/ml significantly induced biphasic SCE levels with approximately 1.6 and 2.3 times that of the negative control, plain RPMI, respectively ($p<0.05$). EEZOR at concentrations of 25, 100 and 200 μg/ml, as well as the solvent control, 0.4% V/V DMSO, did not induce SCE. The positive control, 0.1 μg/ml DXR, significantly increased SCE level by three times that of the plain RPMI ($p<0.05$).

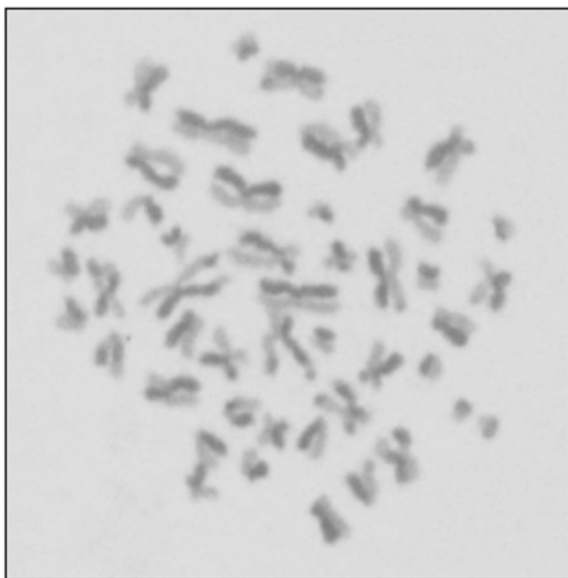

Fig. 2 Heterogeneously stained chromatids in a metaphase cell from the second cell cycle (MII) in control group, plain RPMI.

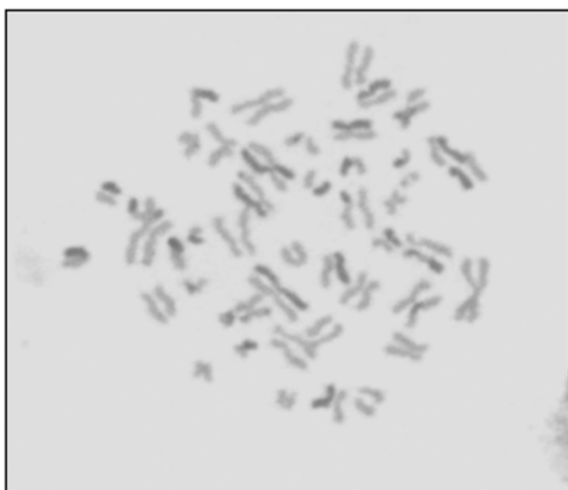

Fig. 3 Mixed homogeneously and heterogenously stained chromatids in a metaphase cell from the third cell cycle (MIII) in control group, plain RPMI.

Cytotoxic activities and effects on cell proliferation by EEZOR

MI and PI of EEZOR treatments at concentrations of 25, 50, 200, 200, 400 μg/ml, including 0.4% V/V DMSO and 0.1 μg/ml DXR, were not significantly different from those of the control RPMI as shown in Table 1. However, the MI and PI values of the EEZOR treatment at concentration of 50 μg/ml were quite low. EEZOR at concentration of 500 μg/ml induced cytotoxicity as few mitotic cells were observed.

Influence of EEZOR on the cell cycle of human lymphocytes

The effect of the EEZOR on cell proliferation was further analyzed and demonstrated that different concentrations of EEZOR affected cell cycle differently (Fig. 5). Biphasic induction of cell cycle delay as shown by the higher number of metaphase cells in the first cell cycle (MI) likely occurred at concentrations of 25 μg/ml and 400 μg/ml EEZOR. From 50 μg/ml to 200 μg/ml EEZOR, there was an almost linear increase in metaphase cells in MI, MII, and MIII suggesting cells

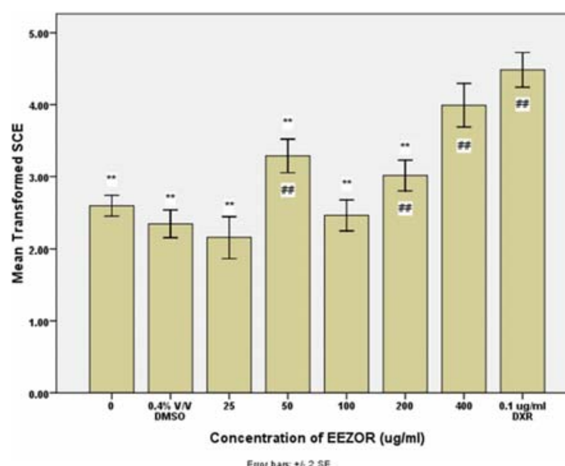

Fig. 4 Sister chromatid exchange levels represented as transformed SCE induced by EEZOR treatments in human lymphocytes in vitro.
$^{##}p<0.05$ indicates a significant difference from the negative control, plain RPMI. $^{**}p<0.05$ indicates a significant difference from the positive control, 0.1 μg/ml doxorubicin (DXR).

Table 1. Mitotic index (MI) and proliferation index (PI) of the EEZOR (Data are shown as means ± standard error)

Concentration of EEZOR (μg/ml)	MI ± SE (n = 3)	PI ± SE (n = 3)
0	12.9±1.0	2.8±0.3
25	15.5±9.8	2.7±1.6
50	4.1±1.0	0.7±0.2
100	8.2±1.8	1.3±0.2
200	12.3±2.9	2.2±0.6
400	8.3±2.8	1.2±0.4
500	Toxic	Toxic
0.4% V/V DMSO	9.8±3.9	1.0±0.9
0.1 mg/ml DXR	10.9±4.9	2.2±1.0

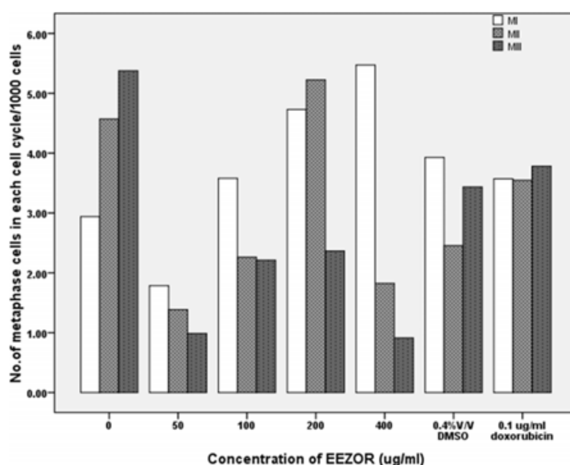

Fig. 5 Influence of the EEZOR treatments on the cell cycle according to the number of metaphase cells in MI, MII and MIII.

were undergoing cell proliferation. In contrast, at 400 μg/ml EEZOR treatment, cell cycle was delayed showing higher number of metaphase cells in MI than those of MII and MIII. For the positive control, DXR; the solvent control 0.4% V/V DMSO; and the negative RPMI control, cells were undergone normal cell proliferation showing higher number of metaphase cells from MII to MIII.

Genotoxic activity of the EEZOR pretreatments followed by DXR

All pretreatments with EEZOR at concentrations of 12.5, 25, 50 and 100 μg/ml, except at 200 μg/ml followed by 0.1 μg/ml DXR treatment, significantly increased SCE level approximately 1.3 times that of the DXR alone or 3.6 times that of the RPMI control ($p<0.05$) (Fig. 6). For 0.4% V/V DMSO solvent control in adjunct to DXR, there was a slight increase in SCE (1.2 times that of the DXR alone), but it was not statistically significant difference from that of the DXR treatment. For the positive control, DXR-induced SCE was about 2.8 times that of the negative RPMI control ($p<0.05$).

Cytotoxic activity of the EEZOR pretreatments followed by DXR

The MI of all EEZOR pre-treatments at concentrations of 12.5, 25, 50, 100 and 200 μg/ml were not significantly different from that of the DXR treatment alone (Table 2). However, the PI of EEZOR treatments at 12.5 and 200 μg/ml were significantly lower than that of plain RPMI ($p<0.05$) suggesting cell proliferations slowed down at these levels.

Influence of the EEZOR pretreatments followed by DXR on the cell cycle

The EEZOR pre-treatments at 12.5, 50, 100, 200 μg/ml, except at 25 μg/ml, followed by 0.1 μg/ml DXR had a tendency to increase number of metaphase cells in MI, more than those in MII and in MIII, suggesting a delay of cell cycle (Fig. 7). EEZOR pre-treatment at 25 μg/ml, although, had higher number of the metaphase cells in MII than in MI, but the number of metaphase cells in MIII became quite low, suggesting a return to cell cycle delay. Additionally, the number of metaphase cells in MIII of the highest dose, 200 μg/ml EEZOR pre-treatment was strikingly low, strongly suggesting that cell cycle was interrupted. Considering cell proliferation, 100 μg/ml EEZOR in adjunct to DXR, appeared to be an effective dose for cell proliferation, with the high number of metaphase cells in MII and MIII.

Compared to the negative and positive controls, RPMI and DXR respectively, cells were growing in normal cell proliferation. Their number of metaphase cells in MII and MIII were higher than those in MI. However, the solvent pre-treatment control, 0.4% V/V DMSO/DXR, had a tendency of cell cycle delay as shown by higher number of metaphase cells in MI than those in MII and MIII.

Discussion and Conclusion

EEZOR alone exhibited biphasic genotoxicity at a low dose of 50 μg/ml and at a high dose of 400 μg/ml ($p<0.05$) almost as a dose dependent manner. These particular doses, tended to induce higher cytotoxicity to cells, decrease cell proliferation and induce the cell

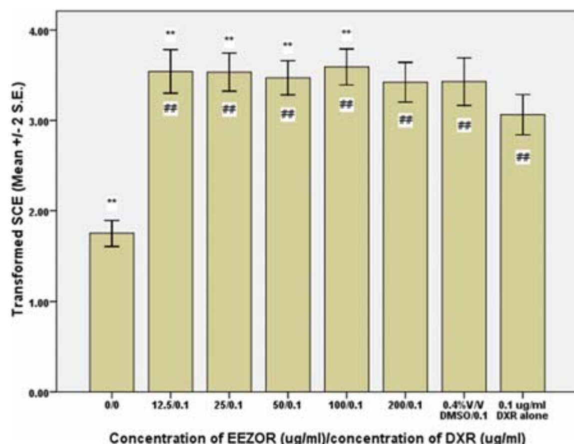

Fig. 6 Sister chromatid exchange levels represented as transformed SCE, induced by EEZOR pretreatments, followed by DXR in human lymphocytes in vitro.
##$p<0.05$ indicates a significant difference from the negative control, plain RPMI alone. **$p<0.05$ indicates a significant difference from the positive control, 0.1 μg/ml doxorubicin (DXR) alone

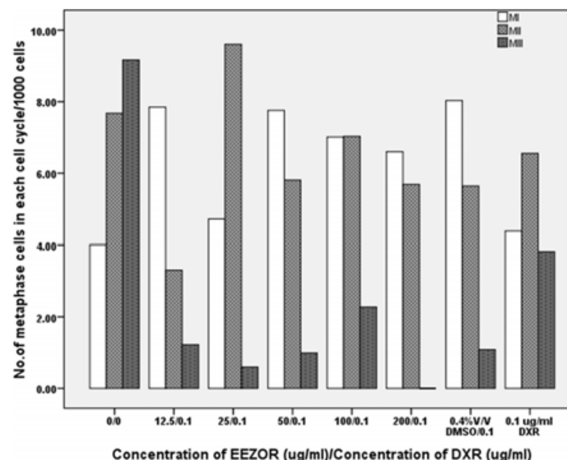

Fig. 7 Influence of the EEZOR pretreatments followed by DXR on the cell cycle according to the number of metaphase cells in MI, MII and MIII.

Table 2. Mitotic index (MI) and proliferation index (PI) of the EEZOR pretreatments followed by DXR (data are shown as means ± standard error)

Concentration of EEZOR pretreatment/DXR (μg/ml)	MI ± SE (n = 3)	PI ± SE (n = 3)
0/0	20.8±2.6	4.7±0.8
12.5/0.1	12.4±2.1	1.8±0.3
25/0.1	14.9±3.8	2.6±0.8
50/0.1	14.6±1.6	2.2±0.3
100/0.1	16.3±5.6	2.8±0.7
200/0.1	12.3±2.6	1.8±0.4**
0.4% V/V DMSO	14.8±2.1	2.2±0.3
0/0.1 (DXR alone)	14.8±5.6	2.9±1.3

** $p<0.05$ significant difference from the negative control (plain RPMI)

cycle delay.

In combination with DXR, EEZOR pretreatments at lower doses of 12.5, 25, 50, and 100 μg/ml significantly enhanced DXR-induced genotoxicity ($p<0.05$). Its solvent, 0.4% V/V DMSO in adjunct to DXR, tended to slightly enhance DXR-induced SCE as well, albeit no statistical significance. Therefore, it is possible that potential of DXR-induced genotoxicity by EEZOR was partially supported by the solvent, DMSO (Fig. 6).

Our finding emphasized that EEZOR alone could be genotoxic to normal human cells depending on its doses. These data are in concordance with the study on prokaryotic cells that 6-gingerol is a potent mutagen when tested against *Escherichia coli*[24]. Nagabhushan et al 1987 indicated that gingerol and shogaol were mutagenic in *Salmonella typhimureum* strain TA100 and TA1535, but zingerone suppressed the mutagenic activities of gingerol and shogaol in a dose dependent manner[25]. Consequently, EEZOR could be both genotoxic and nongenotoxic which might result from their ratio of the bioactive compounds. Previous studies in human cancer cells also reported that 6-gingerol induced genotoxicity in human hepatoma cell line (HepG2) at only high doses but not at low doses[19].

Furthermore, the data suggested that EEZOR induces genotoxicity depending on its doses. Since, the genotoxic dose may not represent cytotoxic dose, and thus it may not induce adverse effect immediately upon the intake. The dosage of EEZOR in humans, therefore, needs to be reconsidered and verified for its long term safety.

In addition, our result demonstrated that EEZOR in combination with DXR could enhance the chemotherapeutic action induced by DXR and induce cell cycle delay. These properties might be responsible for its anticancer by potentiating the genotoxic damage to cancer cells. It might also be responsible for the reduction of drug adverse effects by allowing cells to have more time for cell repair. The ethanolic extract of

ginger has been reported that it can protect various drug-induced toxicity in rats such as thioacetamide-induced liver cirrhosis[26], acetaminophen-induced acute hepatotoxicity[27], and DXR-induced acute nephrotoxicity[28]. Our results, therefore, provide more clarity on a genotoxic mechanism underlying the medicinal activities of EEZOR, and EEZOR in adjunct to DXR, in human lymphocytes, supporting the use of EEZOR in chemotherapy.

Of note, DMSO used as a solvent also has an impact on the experimental results. DMSO itself in adjunct to DXR, slightly enhanced DXR-induced genotoxicity and delayed cell cycle. These could also potentiate the EEZOR activities when EEZOR was used in adjunct to DXR. DMSO is commonly used as a solvent at low doses of less than 2% V/V because of its less toxicity[29]. Anyway, DMSO has been reported to have pharmacological effects such as amelioration of the thioacetamide-induced oxidative stress in rats[30]. In addition, structural studies indicated that DMSO causes cisplatin to bind to a specific protein which could interfere cisplatin toxicity[31]. Therefore, DMSO in our study, although, it was used at a low dose and did not induce genotoxicity by itself, it might directly interfere with EEZOR/DXR actions which could perturb our outcomes. This is our limitation of using DMSO as a solvent. Nonetheless, EEZOR treatments had been performed at various concentrations and the biological responses mostly corresponded to different doses of EEZOR itself, particularly those significantly induction of genotoxicity and cell cycle delay.

In conclusion, our findings suggested that EEZOR in adjunct to DXR is a promising candidate to be used for more effective chemotherapy, despite the fact that its efficacy is limited by its dose. Further in vitro study on mechanistic effects of EEZOR on genetic interaction and in vivo studies of EEZOR/DXR on human cells are needed to clarify the further use of EEZOR to be beneficial in cancer therapy.

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Potential conflicts of interest

None.

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การเสริมฤทธิ์ความเป็นพิษระดับจีนและการปรับวัฏจักรเซลล์ของเซลล์เม็ดเลือดขาวมนุษย์โดยสารสกัดเอทานอลจากขิงร่วมกับยาดอกโซรูบิซินในหลอดทดลอง

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ภูมิหลัง: *การใช้พฤกษเคมีจากธรรมชาติร่วมกับยาเคมีบำบัดเพื่อลดพิษข้างเคียงได้รับความสนใจมากขึ้น ผู้ป่วยที่ได้รับยาดอกโซรูบิซิน (DXR) เมื่อได้รับผลิตภัณฑ์จากเหง้าขิงซิงจิเบอออฟฟีซิเนลด้วย มีรายงานว่าลดอาการคลื่นไส้อาเจียนที่เกิดจากยาได้ นอกจากนั้นยังมีรายงานถึงคุณสมบัติต้านมะเร็งของสารสกัดเอทานอลของเหง้าขิง (EEZOR) อย่างไรก็ตามกลไกการต้านมะเร็งนี้โดยเฉพาะเมื่อใช้ร่วมกับยาเคมีบำบัดยังไม่เป็นที่ทราบแน่ชัด กลไกหนึ่งที่อาจเป็นไปได้คือฤทธิ์ความเป็นพิษระดับจีน*

วัตถุประสงค์: *เพื่อตรวจสอบฤทธิ์ความเป็นพิษระดับจีนและระดับเซลล์ของ EEZOR เดี่ยวและ EEZOR ที่ได้รับก่อนแล้วตามด้วย DXR 0.1 ไมโครกรัม/มิลลิลิตร ต่อเซลล์เม็ดเลือดขาวของมนุษย์ โดยใช้วิธีซิสเตอร์โครมาติดเอ็กซเช้นจ์ แอสเสย์ ในหลอดทดลอง รวมทั้งการตรวจสอบผลต่อจลนศาสตร์ของวัฏจักรเซลล์*

วัสดุและวิธีการ: *นำเม็ดเลือดขาวของมนุษย์มาทดสอบ EEZOR อย่างเดียวที่ความเข้มข้น 25-500 ไมโครกรัม/มิลลิลิตร และ EEZOR ร่วมกับ DXR โดยให้ EEZOR ก่อนที่ความเข้มข้น 12.5-200 ไมโครกรัม/มิลลิลิตร แล้วตามด้วย DXR 0.1 ไมโครกรัม/มิลลิลิตร ประเมินระดับ SCE และจลนศาสตร์ของวัฏจักรเซลล์*

ผลการศึกษา: *EEZOR กระตุ้นให้เกิดการแลกเปลี่ยนชิ้นส่วนโครมาติด (SCE) สูงขึ้นอย่างมีนัยสำคัญ สองระยะคือที่ ความเข้มข้น 50 และ 400 ไมโครกรัม/มิลลิลิตร ($p<0.05$) และกระตุ้นให้เกิดพิษในระดับเซลล์ที่ความเข้มข้น 500 ไมโครกรัม/มิลลิลิตร ส่วน EEZOR ที่ได้รับก่อนให้ DXR นั้นพบว่าเฉพาะ EEZOR ที่ความเข้มข้น 12.5, 25, 50, และ 100 ยกเว้นที่ 200 ไมโครกรัม/มิลลิลิตร สามารถเพิ่มความเป็นพิษของ DXR ที่มีต่อจีนได้ 1.3 เท่า ทั้ง EEZOR เดี่ยวและ EEZOR ร่วมกับ DXR สามารถทำให้วัฏจักรเซลล์ช้าลง*

สรุป: *จากความเข้มข้นที่จำเพาะ EEZOR กระตุ้นการเกิดพิษระดับจีนและหากมีการให้ EEZOR ก่อนแล้วตามด้วย DXR จะกระตุ้นพิษต่อจีนของ DXR ได้มากกว่าเดิมในระดับปานกลาง และทำให้วัฏจักรเซลล์ช้าลง ผลการศึกษาชี้แนะถึงการคำนึงถึงความเข้มข้นของ EEZOR ให้อยู่ระดับปลอดภัยในระยะยาวก่อนนำมาใช้ การใช้ EEZOR ร่วมกับ DXR น่าจะมีศักยภาพและประโยชน์ในการกระตุ้นให้ยาเคมีบำบัดออกฤทธิ์ดีขึ้น การศึกษาต่อไปในสัตว์ทดลองและมนุษย์จะช่วยยืนยันคุณสมบัติเหล่านี้ได้*