

The Indonesian BIOMEDICAL JOURNAL



Volume 15 Number 1
February 2023

Published by:



Secretariat: Prodia Tower 9th Floor
Jl. Kramat Raya No.150,
Jakarta 10430, Indonesia
Tel.: +62-21-3144182
Fax.: +62-21-3144181
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org



#200/M/KPT/2020

Included in:

Scopus[®] DOAJ DIRECTORY OF
OPEN ACCESS JOURNALS



REVIEW ARTICLES

Combining Epigenetic and Immunotherapy in Cancer: Molecular Mechanisms
Meiliana A, Wijaya A

Crucial Triad in Pulp-Dentin Complex Regeneration: Dental Stem Cells, Scaffolds, and Signaling Molecules
Sandra F, Sutanto A, Wulandari W, Lambertus R, Celinna M, Dewi NM, Ichwan SJA

RESEARCH ARTICLES

Well-organized Granuloma Lymphadenitis Tuberculosis Expressed Lower Macrophage Migration Inhibitory Factor (MIF) Score Compared to the Poorly-organized Granuloma
Kadriyan H, Djannah F, Habib P, Cahyawati TD, Siddik N

Hyperbaric Oxygen Ameliorates The Expression of Tumor Growth Factor- β and Malondialdehyde in Pristane-induced Lupus Nephritis Mice Model
Soetjipto, Murbani ID, Harnanik T

Seluang Fish (*Rasbora* sp.) Oil Improves Interleukin-17 Levels and Disease Activity in Rheumatoid Arthritis
Partan RU, Mikhael R, Adinata T, Darma S, Reagan M, Kriswiastiny R, Kusnadi Y, Salim EM

MMP-9 and TIMP-1 Promote Extracellular Matrix Remodeling in the Formation of Ovarian Endometrioma: *in vitro* Study on Chicken Chorioallantoic Membrane
Sari V, Jenie RI, Widad S, Dewanto A

Increased hs-CRP and Sepsis Influence the Occurrence of Thrombocytopenia in Severe and Critically Ill COVID-19 Patients Receiving Anticoagulants
Prayoga AA, Bintoro SUY, Romadhon PZ, Diansyah MN, Amrita PNA, Savitri M, Windradi C, Widiyastuti KN

High Expression of PR-A and Low Expression of PR-B is Correlated with Inflammation in Endometrioma Cases
Yuane E, Dewanto A, Widad S

T118N Substitution of Hepatitis B X Protein Reduces Colony Formation of HepG2 Cells
Artarini A, Nirmalasari DR, Permanasari SC, Riani C, Tjandrawinata RR, Retnoningrum DS

Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid
Ranggaini D, Sandra F, Halim J, Ichwan SJA, Djamil MS

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 1, February 2023

Editor in Chief

Dewi Muliaty, PhD (Prodia Clinical Laboratory, Indonesia)

Managing Editor

Anna Meiliana, PhD (Universitas Padjadjaran, Indonesia)

Board of Editors

Andi Wijaya, PhD (Prodia Clinical Laboratory, Indonesia)
Prof. Dinath Ratnayake (The University of Western Ontario, Canada)
Prof. Geraldine Budomo Dayrit, MSc (University of the Philippines, Philippines)
Prof. Joseph Bercmans Lopez, MSc (MAHSA University College, Malaysia)
Prof. Koichi Nakayama, MD, PhD (Saga University, Japan)
Prof. Rajiv Timothy Erasmus, PhD (Stellenbosch University, South Africa)
Rizky Abdullah, PhD (Universitas Padjadjaran, Indonesia)
Prof. Roberto Volpe, MD, PhD (National Research Council of Italy, Italy)
Prof. Tar Choon Aw, MBBS, Mmed (ICON Central Laboratory, Singapore)
Trilis Yulianti, PhD (Prodia Clinical Laboratory, Indonesia)

Peer Reviewers

Prof. Adekunle Bashiru Okesina, PhD (University of Ilorin Teaching Hospital, Nigeria)
Antonia Anna Lukito, MD, PhD (Universitas Pelita Harapan, Indonesia)
Anwar Santoso, MD, PhD (Universitas Indonesia, Indonesia)
Cynthia Retna Sartika, PhD (Prodia Stem Cell Laboratory, Indonesia)
Prof. Djangan Sargowo, MD, PhD (Universitas Brawijaya, Indonesia)
Elizabeth Henny Herningtyas, MD, PhD (Universitas Gadjah Mada, Indonesia)
Prof. Gerard Pals, PhD (Amsterdam University Medical Center, Netherlands)
Indriyanti Rafi Sukmawati, PhD (Prodia Clinical Laboratory, Indonesia)
Jajah Fachiroh, PhD (Universitas Gadjah Mada, Indonesia)
Prof. Khosrow Adeli, PhD (University of Toronto, Canada)
Laifa A Hendarmin, DDS, PhD (Syarif Hidayatullah State Islamic University, Indonesia)
Marita Kaniawati, PhD (Universitas Bhakti Kencana, Indonesia)
Melisa Intan Barliana, PhD (Universitas Padjadjaran, Indonesia)
Prof. Miki Nakajima, PhD (Kanazawa University, Japan)
Prof. Rahajuningsih D Setiabudy, MD, PhD (Universitas Indonesia, Indonesia)
Raj Kumar Yadav, PhD (Anderson Cancer Center/University of Texas, USA)
Prof. Samuel Vasikaran, MD (Fiona Stanley Hospital, Australia)
Prof. Siti Boedina Kresno, MD, PhD (Universitas Indonesia, Indonesia)
Sunarno, PhD (Ministry of Health of Republic Indonesia, Indonesia)
Yenny Surjawan, MD, PhD (Prodia Clinical Laboratory, Indonesia)

Contact Address

Secretariat of The Indonesian Biomedical Journal

Attn: Nurrani Mustika Dewi, M.Pharm

Prodia Tower 9th Floor

Jl. Kramat Raya No.150, Jakarta 10430, Indonesia

Tel.: +62-21-3144182, ext. 3872

Fax.: +62-21-3144181

WhatsApp No.: +62 877-3616-3117

E-mail: Secretariat@InaBJ.org

Website: www.InaBJ.org

Focus & Scope

The Indonesian Biomedical Journal (InaBJ) is an open access, peer-reviewed journal that encompasses all fundamental and molecular aspects of basic medical sciences, emphasizing on providing the molecular studies of biomedical problems and molecular mechanisms.

InaBJ is dedicated to publish original research and review articles covering all aspects in biomedical sciences. The editors will carefully select manuscript to present only the most recent findings in basic and clinical sciences. All professionals concerned with biomedical issues will find this journal a most valuable update to keep them abreast of the latest scientific development.

Section Policies

Review Article

Review Article should consist of no more than 10,000 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than eight figures and/or tables in total and no more than 250 references. Only invited authors are allowed to submit review article.

Research Article

Research Article should consist of no more than 3,500 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than six figures and/or tables in total and no more than 40 references.

Peer Review Process

All manuscripts submitted to InaBJ will be selected and double-blind peer-reviewed by two or more reviewers to present valuable and authentic findings in biomedical sciences. At least, an external reviewer will be included as the reviewer in each manuscript reviewing process.

Author can suggest reviewer/s that not having publication together within five years and should not be member/s of the same research institution. However, reviewers will be selected independently by Section Editor based on their expertise, specialties, and independencies to fit the topic. Section Editor will ensure that the reviewers will be not from the same institution as the author.

Manuscript will be reviewed comprehensively, including appropriate title; content reflecting abstract; concise writing; clear purpose, study method and figures and/or tables; and summary supported by content. Supplementary data will also be sent to reviewer. The reviewing process will take generally 2-3 months depends on sufficiency of information provided.

Decisions are ultimately made by the Section Editor based on the peer-reviewing results. Therefore, Section Editor will consider thoroughly, if necessary Section Editor can invite another one or more reviewer/s to conclude the final decision.

Publication Frequency

InaBJ is published bimonthly (in February, April, June, August, October, and December).

Open Access Policy

InaBJ provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Content

The Indonesian Biomedical Journal
Volume 15 Number 1, February 2023

REVIEW ARTICLE

Combining Epigenetic and Immunotherapy in Cancer: Molecular Mechanisms

Meiliana A, Wijaya A
p.1-24

Crucial Triad in Pulp-Dentin Complex Regeneration: Dental Stem Cells, Scaffolds, and Signaling Molecules

Sandra F, Sutanto A, Wulandari W, Lambertus R, Celinna M, Dewi NM, Ichwan SJA
p.25-46

RESEARCH ARTICLE

Well-organized Granuloma Lymphadenitis Tuberculosis Expressed Lower Macrophage Migration Inhibitory Factor (MIF) Score Compared to the Poorly-organized Granuloma

Kadriyan H, Djannah F, Habib P, Cahyawati TD, Siddik N
p.47-53

Hyperbaric Oxygen Ameliorates The Expression of Tumor Growth Factor- β and Malondialdehyde in Pristane-induced Lupus Nephritis Mice Model

Soetjipto, Murbani ID, Harnanik T
p.54-60

Seluang Fish (*Rasbora* sp.) Oil Improves Interleukin-17 Levels and Disease Activity in Rheumatoid Arthritis

Partan RU, Mikhael R, Adinata T, Darma S, Reagan M, Kriswiastiny R, Kusnadi Y, Salim EM
p.61-8

RESEARCH ARTICLE

MMP-9 and TIMP-1 Promote Extracellular Matrix Remodeling in the Formation of Ovarian Endometrioma: *in vitro* Study on Chicken Chorioallantoic Membrane

Sari V, Jenie RI, Widad S, Dewanto A
p.69-76

Increased hs-CRP and Sepsis Influence the Occurrence of Thrombocytopenia in Severe and Critically Ill COVID-19 Patients Receiving Anticoagulants

Prayoga AA, Bintoro SUY, Romadhon PZ, Diansyah MN, Amrita PNA, Savitri M, Windradi C, Widiyastuti KN
p.77-84

High Expression of PR-A and Low Expression of PR-B is Correlated with Inflammation in Endometrioma Cases

Yuane E, Dewanto A, Widad S
p.85-93

T118N Substitution of Hepatitis B X Protein Reduces Colony Formation of HepG2 Cells

Artarini A, Nurmalasari DR, Permanasari SC, Riani C, Tjandrawinata RR, Retnoningrum DS
p.94-9

***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid**

Ranggaini D, Sandra F, Halim J, Ichwan SJA, Djamil MS
p.100-5

RESEARCH ARTICLE

Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid

Dewi Ranggaini¹, Ferry Sandra^{2,*}, Johni Halim¹, Solachuddin Jauhari Arief Ichwan³,
Melanie Sadono Djamil²

¹Department of Physiology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

²Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

³Dentistry Programme, PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam

*Corresponding author. E-mail: ferry@trisakti.ac.id

Received date: Jan 6, 2023; Revised date: Jan 19, 2023; Accepted date: Jan 20, 2023

Abstract

BACKGROUND: *Curcuma xanthorrhiza* rhizomes have been demonstrated to have anticancer properties toward various types of cancer cells. The effect of *C. xanthorrhiza* rhizome extract (CXRE) on nasopharyngeal cancer (NPC) cells, including HONE-1 cell line has not been elucidated yet. Therefore, the effect of CXRE on the apoptosis of HONE-1 cells and its possible underlying mechanism are necessary to be explored.

METHODS: *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled ethanol, filtered, and evaporated to produce CXRE. HONE-1 cells were seeded, starved, and treated with dimethyl sulfoxide (DMSO), Doxorubicin, or various concentrations of CXRE. Treated HONE-1 cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) and the number of viable cells was counted. HONE-1 cells were also collected, lysed, and further processed for immunoblotting analysis to measure Bid activity.

RESULTS: The number of viable HONE-1 cells decreased in concentration- and time-dependent manner. The number of viable cells in 50 and 250 µg/mL CXRE-treated groups were significantly lower compared with that in the DMSO-treated group after 24 h. At 48 h incubation period, the number of viable cells in 10, 50 and 250 µg/mL CXRE-treated groups were significantly lower compared with that in the DMSO-treated group. The number of viable cells in 250 µg/mL CXRE-treatment group was not significantly different compared with that in the Doxorubicin-treated group after 48 h. Bid expression levels in CXRE-treated groups were lower compared with that in the DMSO-treated group.

CONCLUSION: CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1 cells.

KEYWORDS: *Curcuma xanthorrhiza*, nasopharyngeal cancer, HONE-1 cells, apoptosis, Bid

Indones Biomed J. 2023; 15(1): 100-5

Introduction

There are 133,354 new nasopharyngeal cancer (NPC) cases and 80,008 deaths worldwide due to this type of cancer in 2020.(1) NPC is considered as the fifth most common cancer in Indonesia after breast, cervix uteri, lung, and liver cancers, with 19,943 new cases and 13,399 deaths.(2) NPC is generally treated with radiotherapy, while

combination of radiotherapy and chemotherapy is used to treat advance-stage NPC.(3-5) Standard treatments have been reported to cause numerous adverse effects, some of which are permanent.(6) Advancement in cancer treatment and the discovery of novel anticancer agents is constantly growing. One of the main focuses of the recent cancer research is the development of anticancer agents from natural substances or their derivatives since they are believed to have a potential to inhibit cancer

development and progression without affecting normal cells.(7,8)

Curcuma xanthorrhiza D.Dietr. is a medicinal plant that belongs to Zingiberaceae family. The rhizome of this plant has been reported to have numerous pharmacological activities, such as antibacterial (9,10), antioxidant (11,12), anti-inflammatory (13,14) and anticancer properties (15). These properties are due to the presence of natural compounds, which are dominated by curcuminoids and terpenoids.(16) Xanthorrhizol, the main compound of *C. xanthorrhiza* that distinguishes this species with other *Curcuma* species, has been demonstrated to show anticancer activities on several types of human cancer cells, including hepatoma (17), oral squamous cell carcinoma (18), promyelocytic leukemia (19), and non-small cell carcinoma.(20)

HONE-1, an NPC cell line, is often used to investigate the cytotoxic effect of compounds obtained from a medicinal plant.(21) This cell line has also been used in research that assesses cytotoxicity of extract obtained from *Curcuma* sp. However, the effect of *C. xanthorrhiza* rhizome extract (CXRE) on NPC cells, including HONE-1 cell line has not been elucidated yet.

The cytotoxicity of compounds found in *C. xanthorrhiza* rhizomes on different types of cancer cells may be related to apoptosis.(17-20,22) One of the signaling pathway that could be activated by these compounds to initiate apoptosis is intrinsic apoptotic pathway, which involves activation of B-cell lymphoma (Bcl)-2 homology 3-interacting domain death agonist (Bid). Apoptotic stimuli induce Bid truncation to form truncated Bid (t-Bid). t-Bid increases mitochondrial membrane permeability, which in turn causes the release of apoptogenic factors from mitochondria to cytoplasm. These apoptogenic factors promote the activation of effector caspases that play a critical role in executing cell death.(19,23) Since *C. xanthorrhiza* has been reported to show anticancer potential in various types of cancer, the effect of CXRE on the apoptosis of HONE-1 NPC cells and its possible underlying mechanism are necessary to be explored.

Methods

Plant Sample Collection and Extraction

C. xanthorrhiza rhizome samples were collected from Bogor, Indonesia. The rhizomes were identified and extracted in PT. Aretha Medika Utama, Bandung, Indonesia. Briefly, *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled 70% ethanol for 24 h at room

temperature, and evaporated. The resulting CXRE was then stored at -20°C.

HONE-1 Cell Culture

HONE-1 cells were cultured in RPMI 1640 without L-Gln (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and 1% Penicillin-Streptomycin-Amphotericin B (Gibco). The cells were maintained in an incubator at 37°C with 5% CO₂.

4',6'-diamidino-2-phenylindole (DAPI) Staining

HONE-1 cells were seeded onto coverslips, treated with/without dimethyl sulfoxide (DMSO), 3 µM Doxorubicin (Dankos Farma, Jakarta, Indonesia), or 10, 50, or 250 µg/mL CXRE for 24 or 48 h. CXRE-treated HONE-1 cells were fixed with 70% ethanol for 3 minutes and washed in PBS. HONE-1 cells were then fixed with 0.1% Triton X-100 for 1 minute and stained by applying 1:100 diluted DAPI (Sigma-Aldrich, St. Louis, USA). HONE-1 cells were evaluated and documented under a fluorescence microscope in three replicates. In each slide, viable cell number was counted by using grids by two independent observers.

Immunoblotting

HONE-1 cells were seeded and treated with/without DMSO, 3 µM Doxorubicin, or 10, 50, or 250 µg/mL CXRE for 6 h. Treated HONE-1 cells were lysed with a lysis buffer containing 10X radio-immunoprecipitation assay (RIPA) buffer (Abcam, Cambridge, UK) and phenylmethanesulfonyl fluoride (Sigma-Aldrich). Twenty µL lysates were sodium dodecyl sulfate-polyacrylamide gel electrophoresed, followed by the transfer onto the polyvinylidene difluoride membrane. Blocking was performed with 5% skim milk, then the sheets were probed with rabbit polyclonal anti-BID antibody (Cell Signaling Technology) diluted 1:1000 in phosphate-buffered saline (PBS). Then, goat anti-rabbit IgG HRP-linked antibody (Cell Signaling Technology) diluted 1:2000 in PBS was added. Immun Star HRP Chemiluminescent Kit (Bio-Rad Laboratories) was used to visualize the bands while Alliance 4.7 (UVItech, Cambridge, UK) was used to capture and quantify the bands.

Data Analyses

Statistical analysis was performed with IBM SPSS Statistics version 26 (IBM Corporation, Armonk, NY, USA). Shapiro-Wilk test was performed to analyze the normality of the data. To analyze the differences of the number of viable cells between groups at 24 and 48 h, Kruskal-Wallis test followed by post hoc Mann-Whitney U test were used.

Results

CXRE Decreased the Amount of Viable HONE-1 Cells

At 24 and 48 h, the viability of HONE-1 cells in the DMSO-treated group was the highest compared with other groups (Figure 1A, 2A), while the viability of HONE-1 cells in the Doxorubicin-treated group was the lowest (Figure 1B, 2B) as indicated by DAPI staining results. There were 337.83 ± 66.58 and 297.50 ± 81.44 viable HONE-1 cells in the DMSO-treated group at 24 and 48 h, respectively. Meanwhile, there were only 7.00 ± 5.87 and 4.83 ± 2.40 viable cells after Doxorubicin treatment for 24 and 48 h, respectively (Figure 3). Upon CXRE addition, the viability of HONE-1 cells was lower compared with that in the DMSO-treated group, implying that CXRE could reduce the viability of HONE-1 cells (Figure 1C-1E, 2C-2E). The number of viable HONE-1 cells decreased in concentration- and time-dependent manner. The number of viable cells in CXRE-treated groups were significantly lower compared with that in DMSO-treated group ($p < 0.05$), except for 10 $\mu\text{g/mL}$ CXRE-treated group at 24 h ($p = 0.109$). However, the number of viable cells in CXRE-treated groups were significantly higher compared with that in Doxorubicin-treated group ($p < 0.05$), except for 250 $\mu\text{g/mL}$ CXRE-treatment group at 48 h ($p = 0.872$). CXRE-treated groups had lower number of viable cells at 48 h than those at 24 h (Figure 3).

CXRE Reduced Bid Expression in HONE-1 Cells

Bid expression levels in 10, 50, and 250 $\mu\text{g/mL}$ CXRE-treated groups were lower compared with that in the DMSO-treated group. CXRE concentration of 250 $\mu\text{g/mL}$ showed greater reduction in Bid expression than the other two concentrations. Bid expression level in HONE-1 cells treated with 50 $\mu\text{g/mL}$ CXRE was slightly higher compared

with those treated with 10 $\mu\text{g/mL}$ CXRE. Bid expression levels in the CXRE-treated groups were higher compared with that in Doxorubicin-treated group. No band was observed in the Doxorubicin-treated group (Figure 4).

Discussion

In the present study, CXRE reduced the viability of HONE-1 cells in concentration- and time-dependent manner, which may be caused by apoptosis induction. A previous study reported that combination of Cisplatin, *C. xanthorrhiza* rhizome ethanolic extract and *Ficus septica* leaves ethanolic extract enhanced apoptosis of human breast cancer cells, as demonstrated by higher cell death percentage when compared with those that were treated with Cisplatin merely.(24) Active compounds found in *C. xanthorrhiza* rhizomes have also been reported to induce apoptosis of several cancer cells. Xanthorrhizol has been demonstrated to promote apoptosis in human hepatoma (17), promyelocytic leukemia (19), and non-small cell carcinoma cells.(20) Curcumin, another important compounds in rhizomes of *C. xanthorrhiza* and other *Curcuma* species (11,25), has been shown to promote apoptosis in NPC cell lines, such as NPC-TW 076 (26), CNE1 and CNE2 (27), as well as other types of cancer, including prostate cancer (22) and acute myeloid leukemia cells.(28) Interestingly, a study reveals that a combination of xanthorrhizol and curcumin synergistically inhibit cell growth by inducing apoptosis in human breast cancer cells.(29)

To confirm whether CXRE promoted apoptosis of HONE-1 cells, the expression levels of Bid were measured. Upon activation of death receptors by apoptotic signals, full length Bid is truncated by cleaved caspase-8 to form truncated Bid (t-Bid), which interconnects intrinsic and extrinsic apoptotic pathways. Hence, upon activation, the amount

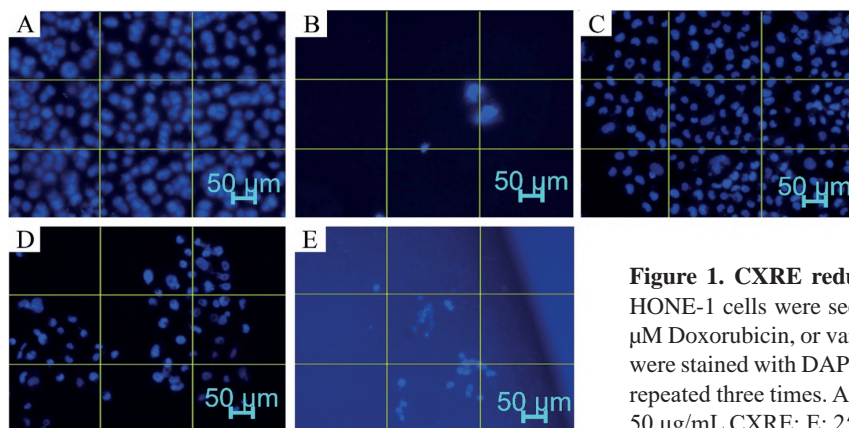


Figure 1. CXRE reduced the viability of HONE-1 cells after 24 h. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μM Doxorubicin, or various concentrations of CXRE for 24 h. Cell nuclei were stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B: Doxorubicin; C: 10 $\mu\text{g/mL}$ CXRE; D: 50 $\mu\text{g/mL}$ CXRE; E: 250 $\mu\text{g/mL}$ CXRE.

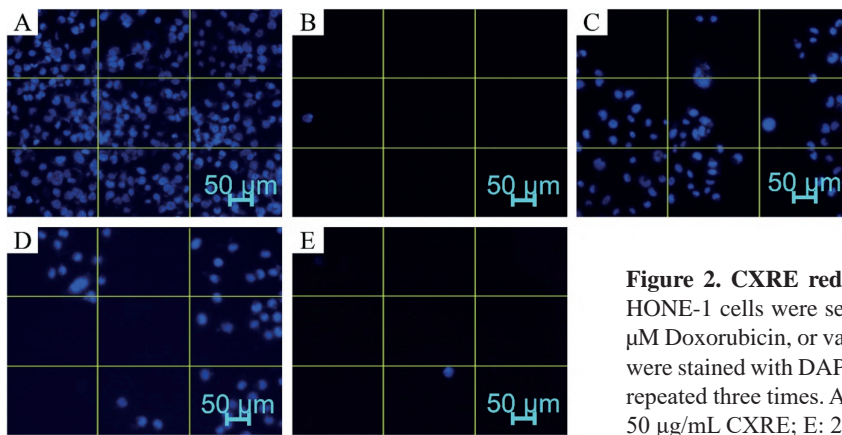


Figure 2. CXRE reduced the viability of HONE-1 cells after 48 h. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or various concentrations of CXRE for 48 h. Cell nuclei were stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B: Doxorubicin; C: 10 μ g/mL CXRE; D: 50 μ g/mL CXRE; E: 250 μ g/mL CXRE.

of Bid is decreased while the amount of t-Bid is increased in the cell. tBid then translocates to mitochondria where it blocks anti-apoptotic activity of Bcl-extra-large (Bcl-XL) and Bcl-2, and activates proapoptotic Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak). This leads to the release of second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis protein-binding protein with low pI (DIABLO) and cytochrome *c*, which play critical roles in executing cell death.(19,23,30) In the present study, Bid expression levels in CXRE-treated HONE-1 cells were lower compared with those in the DMSO-treated group. Thus, it can be concluded that CXRE stimulated Bid activation.

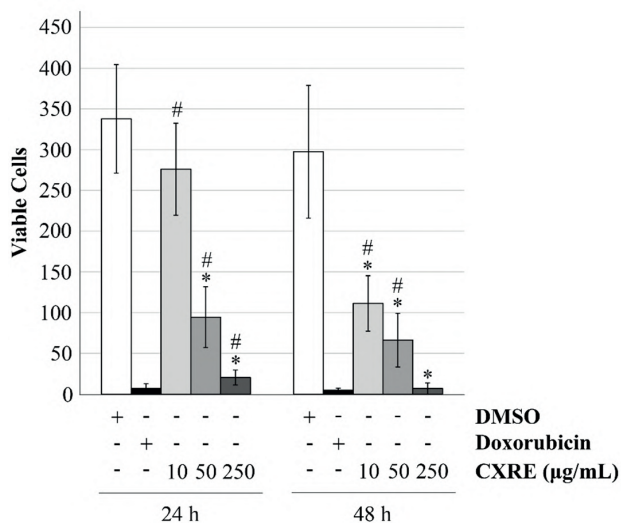


Figure 3. CXRE decreased the number of viable HONE-1 cells in concentration- and time-dependent manner. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or various concentrations of CXRE for 24 and 48 h. Cell nuclei were stained with DAPI. The number of viable cells in each slide was evaluated by two independent observers as described in Methods. The data were expressed as mean \pm SD (n=3). * p <0.05 vs. DMSO-treated group; # p <0.05 vs. Doxorubicin-treated group.

The CXRE-induced Bid truncation could be related to its active compounds, xanthorrhizol and curcumin. Xanthorrhizol has been demonstrated to decrease Bid expression in several types of cancer cells, such as hepatoma (17), promyelocytic leukemia cells (19), and colon cancer (31). Curcumin-induced reduction of Bid expression is also reported in several cancer cells, including glioblastoma (32) and B-precursor acute lymphoblastic leukemia cell lines (33).

Xanthorrhizol has been reported to regulate several signaling pathways which modulate apoptosis induction of cancer cells. Xanthorrhizol inhibits proliferation and induces apoptosis of non-small cell carcinoma cells by inhibiting the activation of phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor kappa B (NF- κ B) pathway, which is involved in controlling cell survival.(20) Interestingly, this compound may also be capable in inducing caspase-independent apoptosis via stimulation of reactive oxygen species (ROS)-

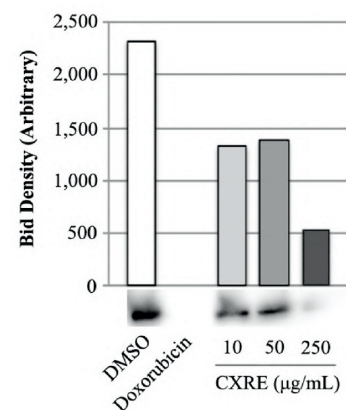


Figure 4. CXRE diminished Bid expression in HONE-1 cells. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or 10, 50, or 250 μ g/mL CXRE for 6 h as indicated in the panel. Cells were collected, lysed, and further processed to obtained cell lysate for Western blot analysis as described in Methods.

mediated p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) in human oral squamous cell carcinoma cells.(18)

Since the results of the present study showed that CXRE activated Bid, which in turn leads HONE-1 cells to apoptosis, components and phenomena in apoptosis signaling pathway both upstream and downstream of Bid, such as DNA fragmentation and $\Delta\Psi_m$ attenuation, as well as the expression level of caspases and apoptogenic factors should be examined.

Conclusion

CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1 cells. Taken together, CXRE is suggested to have cytotoxic effect towards NPC cells, and it could be a potential anticancer agent for NPC.

Authors Contribution

DR and FS prepared study concept and design. DR, FS and JH performed processing and acquisition of data. DR, FS, SJAI and MSD performed analysis and interpretation of results. DR and JH prepared the draft of the manuscript. FS, SJAI and MSD made critical revisions of the manuscript. DR, JH and SJAI assisted in administrative, technical, and material support. FS and MSD performed supervision of the study.

References

- GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020. Nasopharynx, Source: Globocan 2020 [cited 2023 Jan 6]. Available from: <https://gco.iarc.fr/today/data/factsheets/cancers/4-Nasopharynx-fact-sheet.pdf>.
- GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020. Indonesia, Source: Globocan 2020 [cited 2023 Jan 6]. Available from: <https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf>.
- Teo PML, Chan ATC. Treatment strategy and clinical experience. *Semin Cancer Biol.* 2002; 12(6): 497–504.
- Kuhuwael FG, Perkasa MF, Miskad UA, Punagi AQ, Said FA. Comparison of the means of argyrophilic nucleolar organizer region (mAgNOR) pre- and post-therapy in nasopharyngeal carcinoma patients at Wahidin Sudirohusodo General Hospital Makassar. *Indones Biomed J.* 2016; 8(2): 103–8.
- Al Azhar M, Nadliroh S, Prameswari K, Handoko H, Tobing DL, Herawati C. Profile of PD-1 and PD-L1 mRNA expression in peripheral blood of nasopharyngeal carcinoma. *Mol Cell Biomed Sci.* 2020; 4(3): 121–7.
- Vissink A, Jansma J, Spijkervet F, Burlage F, Coppes R. Oral sequelae of head and neck radiotherapy. *Crit Rev Oral Biol Med.* 2003; 14(3): 199–212.
- Sandra F. Targeting ameloblastoma into apoptosis. *Indones Biomed J.* 2018; 10(1): 35–9.
- Novilla A, Mustofa M, Astuti I, Jumina J, Suwito H. Cytotoxic activity of methoxy-4' amino chalcone derivatives against leukemia cell lines. *Mol Cell Biomed Sci.* 2019; 3(1): 34–41.
- Ngadino, Setiawan, Koerniasari, Ernawati, Sudjarwo S. Evaluation of antimycobacterial activity of Curcuma xanthorrhiza ethanolic extract against Mycobacterium tuberculosis H37Rv in vitro. *Vet World.* 2018; 11(3): 368–72.
- Yogiara, Mordukhova EA, Kim D, Kim WG, Hwang JK, Pan JG. The food-grade antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in Escherichia coli. *Bioorg Med Chem Lett.* 2020; 30(24): 127651. doi: 10.1016/j.bmcl.2020.127651.
- Jantan I, Saputri FC, Qaisar MN, Buang F. Correlation between chemical composition of Curcuma domestica and Curcuma xanthorrhiza and their antioxidant effect on human low-density lipoprotein oxidation. *Evid Based Complement Alternat Med.* 2012; 2012: 438356. doi: 10.1155/2012/438356.
- Devaraj S, Ismail S, Ramanathan S, Yam MF. Investigation of antioxidant and hepatoprotective activity of standardized Curcuma xanthorrhiza rhizome in carbon tetrachloride-induced hepatic damaged rats. *ScientificWorldJournal.* 2014; 2014: 353128. doi: 10.1155/2014/353128.
- Kim MB, Kim C, Song Y, Hwang JK. Antihyperglycemic and anti-inflammatory effects of standardized Curcuma xanthorrhiza Roxb. extract and its active compound xanthorrhizol in high-fat diet-induced obese mice. *Evid Based Complement Alternat Med.* 2014; 2014: 205915. doi: 10.1155/2014/205915.
- Kim S, Kook KE, Kim C, Hwang JK. Inhibitory effects of Curcuma xanthorrhiza supercritical extract and xanthorrhizol on LPS-induced inflammation in HGF-1 cells and RANKL-induced osteoclastogenesis in RAW264.7 cells. *J Microbiol Biotechnol.* 2018; 28(8): 1270–81.
- Park JH, Park KK, Kim MJ, Hwang JK, Park SK, Chung WY. Cancer chemoprotective effects of Curcuma xanthorrhiza. *Phytother Res.* 2008; 22(5): 695–8.
- Zhang CM, Fan PH, Li M, Lou HX. Two new sesquiterpenoids from the rhizomes of Curcuma xanthorrhiza. *Helv Chim Acta.* 2014 Sep; 97(9): 1295–300.
- Tee TT, Cheah YH, Meenakshii N, Mohd Sharom MY, Azimahtol Hawariah LP. Xanthorrhizol induced DNA fragmentation in HepG2 cells involving Bcl-2 family proteins. *Biochem Biophys Res Commun.* 2012; 420(4): 834–8.
- Kim JY, An JM, Chung WY, Park KK, Hwang JK, Kim DS, *et al.* Xanthorrhizol induces apoptosis through ROS-mediated MAPK activation in human oral squamous cell carcinoma cells and inhibits DMBA-induced oral carcinogenesis in hamsters. *Phytother Res.* 2013; 27(4): 493–8.
- Kim HJ, Chung WY, Hwang JK, Park KK. Xanthorrhizol induces apoptotic cell death through molecular cross talks between mitochondria-dependent and death receptor-mediated signaling in human promyelocytic leukemia cells. *Cancer Prev Res.* 2013; 18: 41–7.
- Cai Y, Sheng Z, Wang J. Xanthorrhizol inhibits non-small cell carcinoma (A549) cell growth and promotes apoptosis through modulation of PI3K/AKT and NF- κ B signaling pathway. *Environ Toxicol.* 2022; 37(1): 120–30.
- Luo P, Cheng Y, Yin Z, Li C, Xu J, Gu Q. Monomeric and dimeric cytotoxic guaianolide-type sesquiterpenoids from the aerial parts of

- Chrysanthemum indicum*. J Nat Prod. 2019; 82(2): 349–57.
22. Pan L, Sha J, Lin W, Wang Y, Bian T. Curcumin inhibits prostate cancer progression by regulating the miR-30a-5p/PCLAF axis. *Exp Ther Med*. 2021; 22(3): 969. doi: 10.3892/etm.2021.10401.
 23. Lin HF, Hsieh MJ, Hsi YT, Lo YS, Chuang YC, Chen MK, *et al.* Celastrol-induced apoptosis in human nasopharyngeal carcinoma is associated with the activation of the death receptor and the mitochondrial pathway. *Oncol Lett*. 2017; 14(2): 1683–90.
 24. Hidayati DN, Jenie RI, Meiyanto E. Combination of curcuma (*Curcuma xanthorrhiza* Roxb) rhizome ethanolic extract and awar-awar (*Ficus septica* Burm.F) leaves ethanolic extract increases Cisplatin cytotoxicity on T47D breast cancer cells through cell cycle modulation. *Indones J Cancer Chemoprevent*. 2017; 8(3):114–8.
 25. Rahmat E, Lee J, Kang Y. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. *Evid Based Complement Alternat Med*. 2021; 2021: 9960813. doi: 10.1155/2021/9960813.
 26. Kuo C, Wu S, Ip S, Wu P, Yu C, Yang J, *et al.* Apoptotic death in curcumin-treated NPC-TW 076 human nasopharyngeal carcinoma cells is mediated through the ROS, mitochondrial depolarization and caspase-3-dependent signaling responses. *Int J Oncol*. 2011; 39(2): 319–28.
 27. Feng S, Wang Y, Zhang R, Yang G, Liang Z, Wang Z, *et al.* Curcumin exerts its antitumor activity through regulation of miR-7/Skp2/p21 in nasopharyngeal carcinoma cells. *Onco Targets Ther*. 2017; 10: 2377–88.
 28. Zhou H, Ning Y, Zeng G, Zhou C, Ding X. Curcumin promotes cell cycle arrest and apoptosis of acute myeloid leukemia cells by inactivating AKT. *Oncol Rep*. 2021; 45(4): 11. doi: 10.3892/or.2021.7962.
 29. Cheah Y, Nordin F, Sarip R, Tee T, Azimahtol H, Sirat HM, *et al.* Combined xanthorrhizol-curcumin exhibits synergistic growth inhibitory activity via apoptosis induction in human breast cancer cells MDA-MB-231. *Cancer Cell Int*. 2009; 9(1): 1. doi: 10.1186/1475-2867-9-1.
 30. Sandra F, Sidharta MA. Caffeic acid induced apoptosis in MG63 osteosarcoma cells through activation of caspases. *Mol Cell Biomed Sci*. 2017; 1(1): 28–33.
 31. Kang YJ, Park KK, Chung WY, Hwang JK, Lee SK. Xanthorrhizol, a natural sesquiterpenoid, induces apoptosis and growth arrest in HCT116 human colon cancer cells. *J Pharmacol Sci*. 2009; 111(3): 276–84.
 32. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG cells. *Neurochem Res*. 2007; 32(12): 2103–13.
 33. Kuttikrishnan S, Siveen KS, Prabhu KS, Khan AQ, Ahmed EI, Akhtar S, *et al.* Curcumin induces apoptotic cell death via inhibition of PI3-kinase/AKT pathway in B-precursor acute lymphoblastic leukemia. *Front Oncol*. 2019; 9: 484. doi: 10.3389/fonc.2019.00484.



Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author: Ferry Sandra
Assignment title: SIJALI 5
Submission title: InaBJ V15N1A10 - Curcuma xanthorrhiza Rhizome Extract In...
File name: V15N1A10.pdf
File size: 1.58M
Page count: 6
Word count: 3,793
Character count: 20,892
Submission date: 17-Jan-2024 08:39AM (UTC+0700)
Submission ID: 2272236039

The Indonesian Biomedical Journal, Vol.15, No.1, February 2023, p.1-105 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid

Dewi Ranggani¹, Ferry Sandra^{2*}, Johni Halim¹, Solachuddin Jauhari Arief Ichwan³,
Melanie Saldono Djamil¹

¹Department of Physiology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia
²Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia
³Dentistry Programme, PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Jalan Tungku Larik, Gadong BE1410, Brunei Darussalam

*Corresponding author. E-mail: ferry@trisakti.ac.id

Received date: Jan 6, 2023; Revised date: Jan 19, 2023; Accepted date: Jan 20, 2023

Abstract

BACKGROUND: *Curcuma xanthorrhiza* rhizomes have been demonstrated to have anticancer properties toward various types of cancer cells. The effect of *C. xanthorrhiza* rhizome extract (CXRE) on nasopharyngeal cancer (NPC) cells, including HONE-1 cell line has not been elucidated yet. Therefore, the effect of CXRE on the apoptosis of HONE-1 cells and its possible underlying mechanism are necessary to be explored.

METHODS: *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled ethanol, filtered, and evaporated to produce CXRE. HONE-1 cells were seeded, starved, and treated with dimethyl sulfoxide (DMSO), Doxorubicin, or various concentrations of CXRE. Treated HONE-1 cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) and the number of viable cells was counted. HONE-1 cells were also collected, lysed, and further processed for immunoblotting analysis to measure Bid activity.

RESULTS: The number of viable HONE-1 cells decreased in concentration- and time-dependent manner. The number of viable cells in 50 and 250 µg/ml CXRE-treated groups were significantly lower compared with that in the DMSO-treated group after 24 h. At 48 h incubation period, the number of viable cells in 10, 50 and 250 µg/ml CXRE-treated groups were significantly lower compared with that in the DMSO-treated group. The number of viable cells in 250 µg/ml CXRE-treatment group was not significantly different compared with that in the Doxorubicin-treated group after 48 h. Bid expression levels in CXRE-treated groups were lower compared with that in the DMSO-treated group.

CONCLUSION: CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1 cells.

KEYWORDS: *Curcuma xanthorrhiza*, nasopharyngeal cancer, HONE-1 cells, apoptosis, Bid

Indones Biomed J. 2023; 15(1): 100-5

Introduction

There are 133,354 new nasopharyngeal cancer (NPC) cases and 80,008 deaths worldwide due to this type of cancer in 2020.(1) NPC is considered as the fifth most common cancer in Indonesia after breast, cervix uteri, lung, and liver cancers, with 19,943 new cases and 13,399 deaths.(2) NPC is generally treated with radiotherapy, while combination of radiotherapy and chemotherapy is used to treat advance-stage NPC.(3-5) Standard treatments have been reported to cause numerous adverse effects, some of which are permanent.(6) Advancement in cancer treatment and the discovery of novel anticancer agents is constantly growing. One of the main focuses of the recent cancer research is the development of anticancer agents from natural substances or their derivatives since they are believed to have a potential to inhibit cancer

100

Copyright © 2023 The Author(s). All rights reserved.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

InaBJ V15N1A10 - Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid

by Ferry Sandra

Submission date: 17-Jan-2024 08:39AM (UTC+0700)

Submission ID: 2272236039

File name: V15N1A10.pdf (1.58M)

Word count: 3793

Character count: 20892

RESEARCH ARTICLE

***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid**Dewi Ranggaini¹, Ferry Sandra^{2,*}, Johni Halim¹, Solachuddin Jauhari Arief Ichwan³,
Melanie Sadono Djamil²¹Department of Physiology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia²Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia³Dentistry Programme, PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam

*Corresponding author. E-mail: ferry@trisakti.ac.id

Received date: Jan 6, 2023; Revised date: Jan 19, 2023; Accepted date: Jan 20, 2023

Abstract

BACKGROUND: *Curcuma xanthorrhiza* rhizomes have been demonstrated to have anticancer properties toward various types of cancer cells. The effect of *C. xanthorrhiza* rhizome extract (CXRE) on nasopharyngeal cancer (NPC) cells, including HONE-1 cell line has not been elucidated yet. Therefore, the effect of CXRE on the apoptosis of HONE-1 cells and its possible underlying mechanism are necessary to be explored.

METHODS: *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled ethanol, filtered, and evaporated to produce CXRE. HONE-1 cells were seeded, starved, and treated with dimethyl sulfoxide (DMSO), Doxorubicin, or various concentrations of CXRE. Treated HONE-1 cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) and the number of viable cells was counted. HONE-1 cells were also collected, lysed, and further processed for immunoblotting analysis to measure Bid activity.

RESULTS: The number of viable HONE-1 cells decreased in concentration- and time-dependent manner. The number of viable cells in 50 and 250 µg/mL CXRE-treated groups were significantly lower compared with that in the DMSO-treated group after 24 h. At 48 h incubation period, the number of viable cells in 10, 50 and 250 µg/mL CXRE-treated groups were significantly lower compared with that in the DMSO-treated group. The number of viable cells in 250 µg/mL CXRE-treatment group was not significantly different compared with that in the Doxorubicin-treated group after 48 h. Bid expression levels in CXRE-treated groups were lower compared with that in the DMSO-treated group.

CONCLUSION: CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1 cells.

KEYWORDS: *Curcuma xanthorrhiza*, nasopharyngeal cancer, HONE-1 cells, apoptosis, Bid

Indones Biomed J. 2023; 15(1): 100-5

Introduction

There are 133,354 new nasopharyngeal cancer (NPC) cases and 80,008 deaths worldwide due to this type of cancer in 2020.(1) NPC is considered as the fifth most common cancer in Indonesia after breast, cervix uteri, lung, and liver cancers, with 19,943 new cases and 13,399 deaths.(2) NPC is generally treated with radiotherapy, while

combination of radiotherapy and chemotherapy is used to treat advance-stage NPC.(3-5) Standard treatments have been reported to cause numerous adverse effects, some of which are permanent.(6) Advancement in cancer treatment and the discovery of novel anticancer agents is constantly growing. One of the main focuses of the recent cancer research is the development of anticancer agents from natural substances or their derivatives since they are believed to have a potential to inhibit cancer

development and progression without affecting normal cells.(7,8)

Curcuma xanthorrhiza D.Dietr. is a medicinal plant that belongs to Zingiberaceae family. The rhizome of this plant has been reported to have numerous pharmacological activities, such as antibacterial (9,10), antioxidant (11,12), anti-inflammatory (13,14) and anticancer properties (15). These properties are due to the presence of natural compounds, which are dominated by curcuminoids and terpenoids.(16) Xanthorrhizol, the main compound of *C. xanthorrhiza* that distinguishes this species with other *Curcuma* species, has been demonstrated to show anticancer activities on several types of human cancer cells, including hepatoma (17), oral squamous cell carcinoma (18), promyelocytic leukemia (19), and non-small cell carcinoma.(20)

HONE-1, an NPC cell line, is often used to investigate the cytotoxic effect of compounds obtained from a medicinal plant.(21) This cell line has also been used in research that assesses cytotoxicity of extract obtained from *Curcuma* sp. However, the effect of *C. xanthorrhiza* rhizome extract (CXRE) on NPC cells, including HONE-1 cell line has not been elucidated yet.

The cytotoxicity of compounds found in *C. xanthorrhiza* rhizomes on different types of cancer cells may be related to apoptosis.(17-20,22) One of the signaling pathway that could be activated by these compounds to initiate apoptosis is intrinsic apoptotic pathway, which involves activation of B-cell lymphoma (Bcl)-2 homology 3-interacting domain death agonist (Bid). Apoptotic stimuli induce Bid truncation to form truncated Bid (t-Bid). t-Bid increases mitochondrial membrane permeability, which in turn causes the release of apoptogenic factors from mitochondria to cytoplasm. These apoptogenic factors promote the activation of effector caspases that play a critical role in executing cell death.(19,23) Since *C. xanthorrhiza* has been reported to show anticancer potential in various types of cancer, the effect of CXRE on the apoptosis of HONE-1 NPC cells and its possible underlying mechanism are necessary to be explored.

Methods

Plant Sample Collection and Extraction

C. xanthorrhiza rhizome samples were collected from Bogor, Indonesia. The rhizomes were identified and extracted in PT. Aretha Medika Utama, Bandung, Indonesia. Briefly, *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled 70% ethanol for 24 h at room

temperature, and evaporated. The resulting CXRE was then stored at -20°C.

HONE-1 Cell Culture

HONE-1 cells were cultured in RPMI 1640 without L-Gln (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and 1% Penicillin-Streptomycin-Amphotericin B (Gibco). The cells were maintained in an incubator at 37°C with 5% CO₂.

4',6'-diamidino-2-phenylindole (DAPI) Staining

HONE-1 cells were seeded onto coverslips, treated with/without dimethyl sulfoxide (DMSO), 3 µM Doxorubicin (Dankos Farma, Jakarta, Indonesia), or 10, 50, or 250 µg/mL CXRE for 24 or 48 h. CXRE-treated HONE-1 cells were fixed with 70% ethanol for 3 minutes and washed in PBS. HONE-1 cells were then fixed with 0.1% Triton X-100 for 1 minute and stained by applying 1:100 diluted DAPI (Sigma-Aldrich, St. Louis, USA). HONE-1 cells were evaluated and documented under a fluorescence microscope in three replicates. In each slide, viable cell number was counted by using grids by two independent observers.

Immunoblotting

HONE-1 cells were seeded and treated with/without DMSO, 3 µM Doxorubicin, or 10, 50, or 250 µg/mL CXRE for 6 h. Treated HONE-1 cells were lysed with a lysis buffer containing 10X radio-immunoprecipitation assay (RIPA) buffer (Abcam, Cambridge, UK) and phenylmethanesulfonyl fluoride (Sigma-Aldrich). Twenty µL lysates were sodium dodecyl sulfate-polyacrylamide gel electrophoresed, followed by the transfer onto the polyvinylidene difluoride membrane. Blocking was performed with 5% skim milk, then the sheets were probed with rabbit polyclonal anti-BID antibody (Cell Signaling Technology) diluted 1:1000 in phosphate-buffered saline (PBS). Then, goat anti-rabbit IgG HRP-linked antibody (Cell Signaling Technology) diluted 1:2000 in PBS was added. Immun Star HRP Chemiluminescent Kit (Bio-Rad Laboratories) was used to visualize the bands while Alliance 4.7 (UVItech, Cambridge, UK) was used to capture and quantify the bands.

Data Analyses

Statistical analysis was performed with IBM SPSS Statistics version 26 (IBM Corporation, Armonk, NY, USA). Shapiro-Wilk test was performed to analyze the normality of the data. To analyze the differences of the number of viable cells between groups at 24 and 48 h, Kruskal-Wallis test followed by post hoc Mann-Whitney U test were used.

Results

CXRE Decreased the Amount of Viable HONE-1 Cells

At 24 and 48 h, the viability of HONE-1 cells in the DMSO-treated group was the highest compared with other groups (Figure 1A, 2A), while the viability of HONE-1 cells in the Doxorubicin-treated group was the lowest (Figure 1B, 2B) as indicated by DAPI staining results. There were 337.83 ± 66.58 and 297.50 ± 81.44 viable HONE-1 cells in the DMSO-treated group at 24 and 48 h, respectively. Meanwhile, there were only 7.00 ± 5.87 and 4.83 ± 2.40 viable cells after Doxorubicin treatment for 24 and 48 h, respectively (Figure 3). Upon CXRE addition, the viability of HONE-1 cells was lower compared with that in the DMSO-treated group, implying that CXRE could reduce the viability of HONE-1 cells (Figure 1C-1E, 2C-2E). The number of viable HONE-1 cells decreased in concentration- and time-dependent manner. The number of viable cells in CXRE-treated groups were significantly lower compared with that in DMSO-treated group ($p < 0.05$), except for 10 $\mu\text{g/mL}$ CXRE-treated group at 24 h ($p = 0.109$). However, the number of viable cells in CXRE-treated groups were significantly higher compared with that in Doxorubicin-treated group ($p < 0.05$), except for 250 $\mu\text{g/mL}$ CXRE-treatment group at 48 h ($p = 0.872$). CXRE-treated groups had lower number of viable cells at 48 h than those at 24 h (Figure 3).

CXRE Reduced Bid Expression in HONE-1 Cells

Bid expression levels in 10, 50, and 250 $\mu\text{g/mL}$ CXRE-treated groups were lower compared with that in the DMSO-treated group. CXRE concentration of 250 $\mu\text{g/mL}$ showed greater reduction in Bid expression than the other two concentrations. Bid expression level in HONE-1 cells treated with 50 $\mu\text{g/mL}$ CXRE was slightly higher compared

with those treated with 10 $\mu\text{g/mL}$ CXRE. Bid expression levels in the CXRE-treated groups were higher compared with that in Doxorubicin-treated group. No band was observed in the Doxorubicin-treated group (Figure 4).

Discussion

In the present study, CXRE reduced the viability of HONE-1 cells in concentration- and time-dependent manner, which may be caused by apoptosis induction. A previous study reported that combination of Cisplatin, *C. xanthorrhiza* rhizome ethanolic extract and *Ficus septica* leaves ethanolic extract enhanced apoptosis of human breast cancer cells, as demonstrated by higher cell death percentage when compared with those that were treated with Cisplatin merely.(24) Active compounds found in *C. xanthorrhiza* rhizomes have also been reported to induce apoptosis of several cancer cells. Xanthorrhizol has been demonstrated to promote apoptosis in human hepatoma (17), promyelocytic leukemia (19), and non-small cell carcinoma cells.(20) Curcumin, another important compounds in rhizomes of *C. xanthorrhiza* and other *Curcuma* species (11,25), has been shown to promote apoptosis in NPC cell lines, such as NPC-TW 076 (26), CNE1 and CNE2 (27), as well as other types of cancer, including prostate cancer (22) and acute myeloid leukemia cells.(28) Interestingly, a study reveals that a combination of xanthorrhizol and curcumin synergistically inhibit cell growth by inducing apoptosis in human breast cancer cells.(29)

To confirm whether CXRE promoted apoptosis of HONE-1 cells, the expression levels of Bid were measured. Upon activation of death receptors by apoptotic signals, full length Bid is truncated by cleaved caspase-8 to form truncated Bid (t-Bid), which interconnects intrinsic and extrinsic apoptotic pathways. Hence, upon activation, the amount

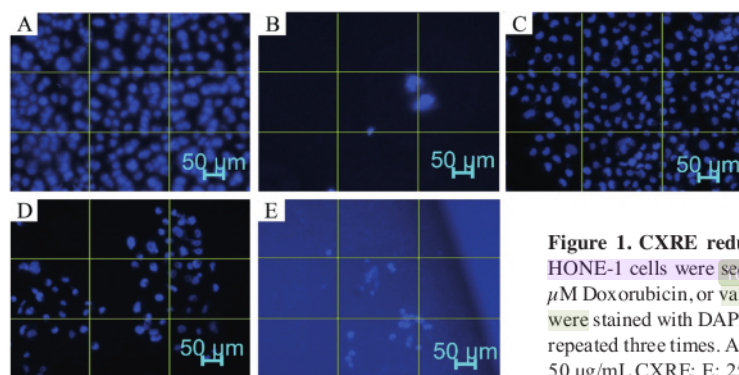


Figure 1. CXRE reduced the viability of HONE-1 cells after 24 h. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μM Doxorubicin, or various concentrations of CXRE for 24 h. Cell nuclei were stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B: Doxorubicin; C: 10 $\mu\text{g/mL}$ CXRE; D: 50 $\mu\text{g/mL}$ CXRE; E: 250 $\mu\text{g/mL}$ CXRE.

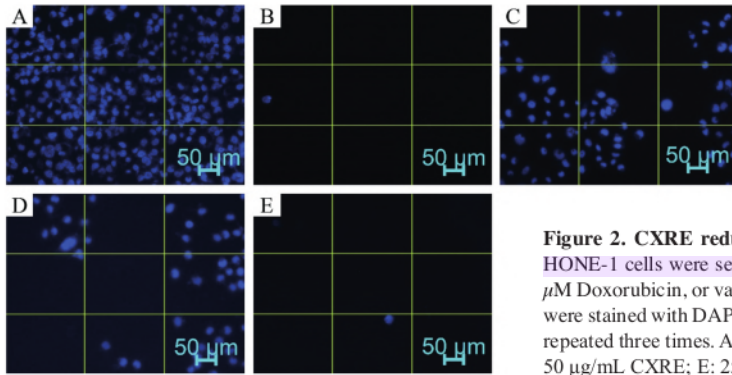


Figure 2. CXRE reduced the viability of HONE-1 cells after 48 h. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or various concentrations of CXRE for 48 h. Cell nuclei were stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B: Doxorubicin; C: 10 μ g/mL CXRE; D: 50 μ g/mL CXRE; E: 250 μ g/mL CXRE.

of Bid is decreased while the amount of t-Bid is increased in the cell. tBid then translocates to mitochondria where it blocks anti-apoptotic activity of Bcl-extra-large (Bcl-XL) and Bcl-2, and activates proapoptotic Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak). This leads to the release of second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis protein-binding protein with low pI (DIABLO) and cytochrome *c*, which play critical roles in executing cell death.(19,23,30) In the present study, Bid expression levels in CXRE-treated HONE-1 cells were lower compared with those in the DMSO-treated group. Thus, it can be concluded that CXRE stimulated Bid activation.

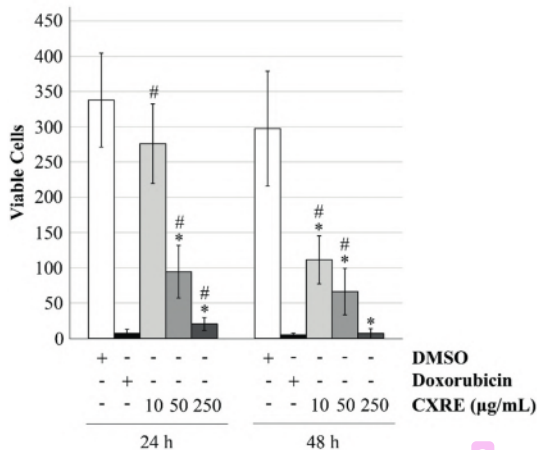


Figure 3. CXRE decreased the number of viable HONE-1 cells in concentration- and time-dependent manner. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or various concentrations of CXRE for 24 and 48 h. Cell nuclei were stained with DAPI. The number of viable cells in each slide was evaluated by two independent observers as described in Methods. The data were expressed as mean \pm SD (n=3). * p <0.05 vs. DMSO-treated group; # p <0.05 vs. Doxorubicin-treated group.

The CXRE-induced Bid truncation could be related to its active compounds, xanthorrhizol and curcumin. Xanthorrhizol has been demonstrated to decrease Bid expression in several types of cancer cells, such as hepatoma (17), promyelocytic leukemia cells (19), and colon cancer (31). Curcumin-induced reduction of Bid expression is also reported in several cancer cells, including glioblastoma (32) and B-precursor acute lymphoblastic leukemia cell lines (33).

Xanthorrhizol has been reported to regulate several signaling pathways which modulate apoptosis induction of cancer cells. Xanthorrhizol inhibits proliferation and induces apoptosis of non-small cell carcinoma cells by inhibiting the activation of phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor kappa B (NF- κ B) pathway, which is involved in controlling cell survival.(20) Interestingly, this compound may also be capable in inducing caspase-independent apoptosis via stimulation of reactive oxygen species (ROS)-

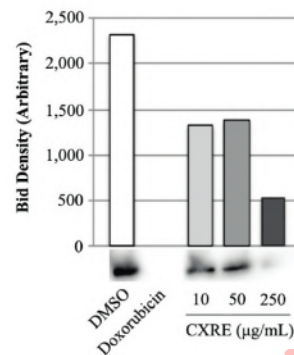


Figure 4. CXRE diminished Bid expression in HONE-1 cells. HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 μ M Doxorubicin, or 10, 50, or 250 μ g/mL CXRE for 6 h as indicated in the panel. Cells were collected, lysed, and further processed to obtain cell lysate for Western blot analysis as described in Methods.

mediated p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) in human oral squamous cell carcinoma cells.(18)

Since the results of the present study showed that CXRE activated Bid, which in turn leads HONE-1 cells to apoptosis, components and phenomena in apoptosis signaling pathway both upstream and downstream of Bid, such as DNA fragmentation and $\Delta\Psi_m$ attenuation, as well as the expression level of caspases and apoptogenic factors should be examined.

Conclusion

CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1 cells. Taken together, CXRE is suggested to have cytotoxic effect towards NPC cells, and it could be a potential anticancer agent for NPC.

Authors Contribution

DR and FS prepared study concept and design. DR, FS and JH performed processing and acquisition of data. DR, FS, SJAI and MSD performed analysis and interpretation of results. DR and JH prepared the draft of the manuscript. FS, SJAI and MSD made critical revisions of the manuscript. DR, JH and SJAI assisted in administrative, technical, and material support. FS and MSD performed supervision of the study.

References

- GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020. Nasopharynx, Source: Globocan 2020 [cited 2023 Jan 6]. Available from: <https://gco.iarc.fr/today/data/factsheets/cancers/4-Nasopharynx-fact-sheet.pdf>.
- GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020. Indonesia, Source: Globocan 2020 [cited 2023 Jan 6]. Available from: <https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf>.
- Teo PML, Chan ATC. Treatment strategy and clinical experience. *Semin Cancer Biol.* 2002; 12(6): 497–504.
- Kuhuwael FG, Perkasa MF, Miskad UA, Punagi AQ, Said FA. Comparison of the means of argyrophilic nucleolar organizer region (mAgNOR) pre- and post-therapy in nasopharyngeal carcinoma patients at Wahidin Sudirohusodo General Hospital Makassar. *Indones Biomed J.* 2016; 8(2): 103–8.
- Al Azhar M, Nadliroh S, Prameswari K, Handoko H, Tobing DL, Herawati C. Profile of PD-1 and PD-L1 mRNA expression in peripheral blood of nasopharyngeal carcinoma. *Mol Cell Biomed Sci.* 2020; 4(3): 121–7.
- Vissink A, Jansma J, Spijkervet F, Burlage F, Coppes R. Oral sequelae of head and neck radiotherapy. *Crit Rev Oral Biol Med.* 2003; 14(3): 199–212.
- Sandra F. Targeting ameloblastoma into apoptosis. *Indones Biomed J.* 2018; 10(1): 35–9.
- Novilla A, Mustofa M, Astuti I, Jumina J, Suwito H. Cytotoxic activity of methoxy-4' amino chalcone derivatives against leukemia cell lines. *Mol Cell Biomed Sci.* 2019; 3(1): 34–41.
- Ngadino, Setiawan, Koerniasari, Ernawati, Sudjarwo S. Evaluation of antimycobacterial activity of Curcuma xanthorrhiza ethanolic extract against Mycobacterium tuberculosis H37Rv in vitro. *Vet World.* 2018; 11(3): 368–72.
- Yogiara, Mordukhova EA, Kim D, Kim WG, Hwang JK, Pan JG. The food-grade antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in Escherichia coli. *Bioorg Med Chem Lett.* 2020; 30(24): 127651. doi: 10.1016/j.bmcl.2020.127651.
- Jantan I, Saputri FC, Qaisar MN, Buang F. Correlation between chemical composition of Curcuma domestica and Curcuma xanthorrhiza and their antioxidant effect on human low-density lipoprotein oxidation. *Evid Based Complement Alternat Med.* 2012; 2012: 438356. doi: 10.1155/2012/438356.
- Devaraj S, Ismail S, Ramanathan S, Yam MF. Investigation of antioxidant and hepatoprotective activity of standardized Curcuma xanthorrhiza rhizome in carbon tetrachloride-induced hepatic damaged rats. *ScientificWorldJournal.* 2014; 2014: 353128. doi: 10.1155/2014/353128.
- Kim MB, Kim C, Song Y, Hwang JK. Antihyperglycemic and anti-inflammatory effects of standardized Curcuma xanthorrhiza Roxb. extract and its active compound xanthorrhizol in high-fat diet-induced obese mice. *Evid Based Complement Alternat Med.* 2014; 2014: 205915. doi: 10.1155/2014/205915.
- Kim S, Kook KE, Kim C, Hwang JK. Inhibitory effects of Curcuma xanthorrhiza supercritical extract and xanthorrhizol on LPS-induced inflammation in HGF-1 cells and RANKL-induced osteoclastogenesis in RAW264.7 cells. *J Microbiol Biotechnol.* 2018; 28(8): 1270–81.
- Park JH, Park KK, Kim MJ, Hwang JK, Park SK, Chung WY. Cancer chemoprotective effects of Curcuma xanthorrhiza. *Phytother Res.* 2008; 22(5): 695–8.
- Zhang CM, Fan PH, Li M, Lou HX. Two new sesquiterpenoids from the rhizomes of Curcuma xanthorrhiza. *Helv Chim Acta.* 2014 Sep; 97(9): 1295–300.
- Tee TT, Cheah YH, Meenakshii N, Mohd Sharom MY, Azimahtol Hawariah LP. Xanthorrhizol induced DNA fragmentation in HepG2 cells involving Bcl-2 family proteins. *Biochem Biophys Res Commun.* 2012; 420(4): 834–8.
- Kim JY, An JM, Chung WY, Park KK, Hwang JK, Kim DS, et al. Xanthorrhizol induces apoptosis through ROS-mediated MAPK activation in human oral squamous cell carcinoma cells and inhibits DMBA-induced oral carcinogenesis in hamsters. *Phytother Res.* 2013; 27(4): 493–8.
- Kim HJ, Chung WY, Hwang JK, Park KK. Xanthorrhizol induces apoptotic cell death through molecular cross talks between mitochondria-dependent and death receptor-mediated signaling in human promyelocytic leukemia cells. *Cancer Prev Res.* 2013; 18: 41–7.
- Cai Y, Sheng Z, Wang J. Xanthorrhizol inhibits non-small cell carcinoma (A549) cell growth and promotes apoptosis through modulation of PI3K/AKT and NF- κ B signaling pathway. *Environ Toxicol.* 2022; 37(1): 120–30.
- Luo P, Cheng Y, Yin Z, Li C, Xu J, Gu Q. Monomeric and dimeric cytotoxic guaianolide-type sesquiterpenoids from the aerial parts of

- Chrysanthemum indicum. *J Nat Prod.* 2019; 82(2): 349–57.
22. Pan L, Sha J, Lin W, Wang Y, Bian T. Curcumin inhibits prostate cancer progression by regulating the miR-30a-5p/PCLAF axis. *Exp Ther Med.* 2021; 22(3): 969. doi: 10.3892/etm.2021.10401.
 23. Lin HF, Hsieh MJ, Hsi YT, Lo YS, Chuang YC, Chen MK, *et al.* Celastrol-induced apoptosis in human nasopharyngeal carcinoma is associated with the activation of the death receptor and the mitochondrial pathway. *Oncol Lett.* 2017; 14(2): 1683–90.
 24. Hidayati DN, Jenie RI, Meiyanto E. Combination of curcuma (*Curcuma xanthorrhiza* Roxb) rhizome ethanolic extract and awarawar (*Ficus septica* Burm.F) leaves ethanolic extract increases Cisplatin cytotoxicity on T47D breast cancer cells through cell cycle modulation. *Indones J Cancer Chemoprevent.* 2017; 8(3):114–8.
 25. Rahmat E, Lee J, Kang Y. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. *Evid Based Complement Alternat Med.* 2021; 2021: 9960813. doi: 10.1155/2021/9960813.
 26. Kuo C, Wu S, Ip S, Wu P, Yu C, Yang J, *et al.* Apoptotic death in curcumin-treated NPC-TW 076 human nasopharyngeal carcinoma cells is mediated through the ROS, mitochondrial depolarization and caspase-3-dependent signaling responses. *Int J Oncol.* 2011; 39(2): 319–28.
 27. Feng S, Wang Y, Zhang R, Yang G, Liang Z, Wang Z, *et al.* Curcumin exerts its antitumor activity through regulation of miR-7/Skp2/p21 in nasopharyngeal carcinoma cells. *Onco Targets Ther.* 2017; 10: 2377–88.
 28. Zhou H, Ning Y, Zeng G, Zhou C, Ding X. Curcumin promotes cell cycle arrest and apoptosis of acute myeloid leukemia cells by inactivating AKT. *Oncol Rep.* 2021; 45(4): 11. doi: 10.3892/or.2021.7962.
 29. Cheah Y, Nordin F, Sarip R, Tee T, Azimahtol H, Sirat HM, *et al.* Combined xanthorrhizol-curcumin exhibits synergistic growth inhibitory activity via apoptosis induction in human breast cancer cells MDA-MB-231. *Cancer Cell Int.* 2009; 9(1): 1. doi: 10.1186/1475-2867-9-1.
 30. Sandra F, Sidharta MA. Caffeic acid induced apoptosis in MG63 osteosarcoma cells through activation of caspases. *Mol Cell Biomed Sci.* 2017; 1(1): 28–33.
 31. Kang YJ, Park KK, Chung WY, Hwang JK, Lee SK. Xanthorrhizol, a natural sesquiterpenoid, induces apoptosis and growth arrest in HCT116 human colon cancer cells. *J Pharmacol Sci.* 2009; 111(3): 276–84.
 32. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG cells. *Neurochem Res.* 2007; 32(12): 2103–13.
 33. Kuttikrishnan S, Siveen KS, Prabhu KS, Khan AQ, Ahmed EI, Akhtar S, *et al.* Curcumin induces apoptotic cell death via inhibition of PI3-kinase/AKT pathway in B-precursor acute lymphoblastic leukemia. *Front Oncol.* 2019; 9: 484. doi: 10.3389/fonc.2019.00484.

InaBJ V15N1A10 - Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid

ORIGINALITY REPORT

10%

SIMILARITY INDEX

5%

INTERNET SOURCES

11%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

- 1 K.M. Li, X. Sun, H.K. Koon, W.N. Leung, M.C. Fung, R.N.S. Wong, Maria L. Lung, C.K. Chang, N.K. Mak. "Apoptosis and expression of cytokines triggered by pyropheophorbide-a methyl ester-mediated photodynamic therapy in nasopharyngeal carcinoma cells", *Photodiagnosis and Photodynamic Therapy*, 2006
Publication 1%
- 2 Chen, K.L.. "Targeting cathepsin S induces tumor cell autophagy via the EGFR-ERK signaling pathway", *Cancer Letters*, 20120401
Publication 1%
- 3 Yee-Man Lee, Choi-Man Ting, Yuen-Kit Cheng, Tai-Ping Fan, Ricky Ngok-Shun Wong, Maria Li Lung, Nai-Ki Mak. "Mechanisms of 2-methoxyestradiol-induced apoptosis and G2/M cell-cycle arrest of nasopharyngeal carcinoma cells", *Cancer Letters*, 2008
Publication 1%

4

Jing-Gung Chung. "Apoptotic death in curcumin-treated NPC-TW 076 human nasopharyngeal carcinoma cells is mediated through the ROS, mitochondrial depolarization and caspase-3-dependent signaling responses", *International Journal of Oncology*, 2011

Publication

1 %

5

smartech.gatech.edu

Internet Source

1 %

6

Siddavaram Nagini, Fabrizio Palitti, Adayapalam T. Natarajan. "Chemopreventive Potential of Chlorophyllin: A Review of the Mechanisms of Action and Molecular Targets", *Nutrition and Cancer*, 2015

Publication

1 %

7

www.frontiersin.org

Internet Source

1 %

8

Zhang, Letian. "RB1 Loss Accelerates acquired Therapeutic Resistance in EGFR Mutant Lung Adenocarcinoma via Lineage Plasticity", State University of New York at Buffalo, 2021

Publication

1 %

9

www.nature.com

Internet Source

1 %

10

tessera.spandidos-publications.com

Internet Source

1 %

11

jeccr.biomedcentral.com

Internet Source

1 %

12

pubmed.ncbi.nlm.nih.gov

Internet Source

1 %

Exclude quotes On

Exclude matches < 15 words

Exclude bibliography On

InaBJ V15N1A10 - Curcuma xanthorrhiza Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid

GRADEMARK REPORT

FINAL GRADE

GENERAL COMMENTS

/15

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6



Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023009 Editor Decision Round 1 - Revisions Required

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: ferry@trisakti.ac.id

Tue, 17 Jan, 2023 at 11:46 AM

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid Activation**".

Our decision is: **Revisions Required.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **January 31, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: <https://inabj.org/index.php/ibj/author/submissionReview/2217>, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

Jl. Kramat Raya No.150, Jakarta 10430, Indonesia

Phone. +62-21-3144182 ext. 3872

Fax. +62-21-3144181

<https://www.inabj.org>

3 attachments

 **Round 1 Reviewer 1 - F09 Manuscript Review Form.pdf**
176K

 **Round 1 Reviewer 2 - F09 Manuscript Review Form.pdf**
693K

 **Round 1 Reviewer 2 - Manuscript.docx**
712K



Manuscript Review Form

Reviewer	: Reviewer 1
Manuscript #	: M2023009
Manuscript Title	: <i>Curcuma xanthorrhiza</i> Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid Activation

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?	V	
	Notes:		
2.	Are the title and abstract of the manuscript appropriate?	V	
	Notes: The abstract is clear, written in a good English structure but can be made more interesting		
3	Do the title and abstract reflect the study result/content?	V	
	Notes:		
4.	Is the significance of the study well explained at the Background?	V	
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?	V	
	Notes: The number of viable cells in each slide was counted by two independent observers. However, the cell count has not been clearly described		



6.	Are the results, ideas, and data presented in this manuscript important enough for publication?	V	
	Notes:		
7.	Are all figures and tables necessarily presented?	V	
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	V	
	Notes: Xanthorrhizol has been demonstrated to decrease Bid expression in several types of cancer cells, such as hepatoma (17), promyelocytic leukemia cells (19), and colon cancer.(31) Curcumin-induced reduction of Bid expression is also reported in several cancer cells, including glioblastoma (32) and B-precursor acute lymphoblastic leukemia cell lines.(33). Please recheck the references, In order to explain further about its apoptotic pathway.		
9.	Are the conclusions and interpretations valid and supported by the data?	V	
	Notes:		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?	V	
	Notes: The manuscript is clear, but can it be made to be more interesting, and written in a good English structure?		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)



The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia

Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181

Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	
Accept Submission (No significant alterations suggested)	<input type="checkbox"/>
Revisions Required (Suggest changes to the manuscript as specified in this review)	<input checked="" type="checkbox"/>
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	<input type="checkbox"/>
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	<input type="checkbox"/>

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Date and Sign:

January 10, 2023

Reviewer 1



Manuscript Review Form

Reviewer	: Reviewer 2
Manuscript #	: M2023009
Manuscript Title	: <i>Curcuma xanthorrhiza</i> Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid Activation

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Notes: This study explored effect of CXRE on the apoptosis of HONE-1 cells via Bid expression		
2.	Are the title and abstract of the manuscript appropriate?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Notes:		
3.	Do the title and abstract reflect the study result/content?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Notes:		
4.	Is the significance of the study well explained at the Background?	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Notes:		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Notes: 1) Why are HONE-1 cells starved for 12 hours prior to treatment? 2) Why use DMSO as a HONE-1 cells therapy agent? What is the concentration of DMSO used in this study? 3) Why use DAPI staining to identify viable cells? DAPI staining (nuclei stained) is applied to observe changes in nuclear morphology (condensed and fragmented nuclei). Cell numbers were reduced due to nuclear shrinkage, chromosome condensation and apoptotic bodies. 4) Why is the HONE-1 cell treatment for the Bid expression carried out only for 6 hours?		
6.	Are the results, ideas, and data presented in this manuscript important enough for publication?	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Notes: 1) The data had large standard deviation, what were this caused by? 2) Lines 125-126: "the number of viable cells in 250 µg/mL CXRE-treatment group		



	was not significantly different compared to that in the Doxorubicin-treated group”. ➤ Why are no bands observed on Doxorubicin-treated group?		
7.	Are all figures and tables necessarily presented?	✓	
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	✓	
	Notes: Lines 154-155: “Bid activation reduces the amount of Bid” ➤ Caspases-8/-10 cleaves Bid to produce tBid, which activates a crosstalk pathway between death receptors and mitochondria. ➤ tBid translocates to mitochondria where it blocks anti-apoptotic activity of Bcl-2 and Bcl-XL, and activates Bax and Bak. ➤ This leads to release of cytochrome c and Smac/Diablo and activation of the mitochondrial pathway of apoptosis.		
9.	Are the conclusions and interpretations valid and supported by the data?	✓	
	Notes:		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?	✓	
	Notes:		

Specific Reviewer’s Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

- It is necessary to analyze the effect of CXRE on growth, proliferation, or apoptosis of normal cell

Reviewer’s Recommendation (Please tick only one option)	✓
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	✓
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	



The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia

Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181

Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Further Reviewer's Comments Regarding Disposition of the Manuscript:

- This is a good paper with the potential to achieve a substantial contribution

Date and Sign:

January 14th, 2023

Reviewer 2

1 ***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal**
2 **Cancer Cells Through Bid Activation**

3
4 **Abstract**

5 **Background:** *Curcuma xanthorrhiza* rhizomes have been demonstrated to have anticancer
6 properties toward various types of cancer cells. The effect of *C. xanthorrhiza* rhizome extract
7 (CXRE) on nasopharyngeal cancer (NPC) cells, including HONE-1 cell line has not been
8 elucidated yet. Therefore, the effect of CXRE on the apoptosis of HONE-1 cells and its
9 possible underlying mechanism are necessary to be explored.

10 **Methods:** *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled ethanol,
11 filtered, and evaporated to produce CXRE. HONE-1 cells were seeded, starved, and treated
12 with dimethyl sulfoxide (DMSO), Doxorubicin, or various concentrations of CXRE. Treated
13 HONE-1 cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) and the number of
14 viable cells was counted. HONE-1 cells were also collected, lysed, and further processed for
15 immunoblotting analysis to measure Bid expression.

16 **Results:** The number of viable HONE-1 cells decreased in concentration- and time-
17 dependent manner. The number of viable cells in 50 and 250 µg/mL CXRE-treated groups
18 were significantly lower compared with that in the DMSO-treated group after 24 h. At 48 h
19 incubation period, the number of viable cells in 10, 50 and 250 µg/mL CXRE-treated groups
20 were significantly lower compared with that in the DMSO-treated group. The number of
21 viable cells in 250 µg/mL CXRE-treatment group was not significantly different compared to
22 that in the Doxorubicin-treated group after 48 h. Bid expression levels in CXRE-treated
23 groups were lower compared with that in the DMSO-treated group.

24 **Conclusion:** CXRE could induce apoptosis via Bid activation, hence reducing the viability of
25 HONE-1 cells.

26 **Keywords:** *Curcuma xanthorrhiza*, nasopharyngeal cancer, HONE-1 cells, apoptosis, Bid

27

28 **Introduction**

29 There are 133,354 new nasopharyngeal cancer (NPC) cases and 80,008 deaths worldwide due
30 to this type of cancer in 2020.(1) NPC is considered as the fifth most common cancer in
31 Indonesia after breast, cervix uteri, lung, and liver cancers, with 19,943 new cases and 13,399
32 deaths.(2) NPC is generally treated with radiotherapy, while combination of radiotherapy and
33 chemotherapy is used to treat advance-stage NPC.(3–5) The standard treatments have been
34 reported to cause numerous adverse effects, some of which are permanent.(6) Advancement
35 in cancer treatment and the discovery of novel anticancer agents is constantly growing. One
36 of the main focuses of the recent cancer research is the development of anticancer agents
37 from natural substances or their derivatives since they are believed to have a potential to
38 inhibit cancer development and progression without affecting normal cells.(7,8)

39 *Curcuma xanthorrhiza* D.Dietr. is a medicinal plant that belongs to Zingiberaceae
40 family. The rhizome of this plant has been reported to have numerous pharmacological
41 activities, such as antibacterial (9,10), antioxidant (11,12), anti-inflammatory (13,14) and
42 anticancer properties.(15) These properties are due to the presence of natural compounds,
43 which are dominated by curcuminoids and terpenoids.(16) Xanthorrhizol, the main
44 compound of *C. xanthorrhiza* that distinguishes this species with other *Curcuma* species, has
45 been demonstrated to show anticancer activities on several types of human cancer cells,
46 including hepatoma (17), oral squamous cell carcinoma (18), promyelocytic leukemia (19),
47 and non-small cell carcinoma.(20)

48 HONE-1, an NPC cell line, is often used to investigate the cytotoxic effect of
49 compounds obtained from a medicinal plant.(21) This cell line has also been used in research
50 that assesses cytotoxicity of extract obtained from *Curcuma* sp. However, the effect of *C.*

51 *xanthorrhiza* rhizome extract (CXRE) on NPC cells, including HONE-1 cell line has not been
52 elucidated yet.

53 The cytotoxicity of compounds found in *C. xanthorrhiza* rhizomes on different types
54 of cancer cells may be related to apoptosis.(17–20,22) One of the signaling pathway that
55 could be activated by these compounds to initiate apoptosis is intrinsic apoptotic pathway,
56 which involves activation of B cell lymphoma 2 homology 3-interacting domain death
57 agonist (Bid). Apoptotic stimuli induce Bid truncation to form truncated Bid (t-Bid). t-Bid
58 increases mitochondrial membrane permeability, which in turn causes the release of
59 apoptogenic factors from mitochondria to cytoplasm. These apoptogenic factors promote the
60 activation of effector caspases that play a critical role in executing cell death.(19,23) Since *C.*
61 *xanthorrhiza* has been reported to show anticancer potential in various types of cancer, the
62 effect of CXRE on the apoptosis of HONE-1 NPC cells and its possible underlying
63 mechanism are necessary to be explored.

64

65 **Methods**

66 **Plant Sample Collection and Extraction**

67 *C. xanthorrhiza* rhizome samples were collected from Bogor, Indonesia. The rhizomes were
68 identified and extracted in Biomolecular Biomedical Research Center, PT. Aretha Medika
69 Utama, Bandung, Indonesia. Briefly, *C. xanthorrhiza* rhizomes were minced and dried. The
70 dried material was extracted with distilled 70% ethanol at room temperature for 24 h, filtered,
71 and evaporated using a rotatory evaporator. The resulting CXRE was then stored at -20°C.

72 **HONE-1 Cell Culture**

73 HONE-1 cells were cultured in RPMI 1640 without L-Gln (Gibco, Grand Island, NY, USA)
74 supplemented with 10% fetal bovine serum (Gibco) and 1% Penicillin-Streptomycin-
75 Amphotericin B (Gibco). The cells were maintained in an incubator at 37°C with 5% CO₂.

76 **4',6'-diamidino-2-phenylindole (DAPI) Staining**

77 HONE-1 cells were seeded onto coverslips, treated with/without dimethyl sulfoxide (DMSO),
78 3 μ M Doxorubicin (Dankos Farma, Jakarta, Indonesia), or 10, 50, or 250 μ g/mL CXRE for
79 24 or 48 h. CXRE-treated HONE-1 cells were fixed with 70% ethanol for 3 minutes and
80 washed in PBS. HONE-1 cells were then fixed with 0.1% Triton X-100 for 1 minute and
81 stained by applying 1:100 diluted DAPI (Sigma-Aldrich, St. Louis, USA). HONE-1 cells
82 were evaluated and documented under a fluorescence microscope. DAPI staining was
83 performed in three replicates and the number of viable cells in each slide was counted by two
84 independent observers.

Comment [BK1]: 1) Why use DMSO as a HONE-1 cells therapy agent?
2) What is the concentration of DMSO used in this study?

85 **Immunoblotting**

86 HONE-1 cells were seeded to 96-well plate and treated with/without DMSO, 3 μ M
87 Doxorubicin, or 10, 50, or 250 μ g/mL CXRE for 6 h. Treated HONE-1 cells were lysed with
88 a lysis buffer containing 10X radio-immunoprecipitation assay (RIPA) buffer (Abcam,
89 Cambridge, UK) and phenylmethanesulfonyl fluoride (Sigma-Aldrich). Twenty μ L lysates
90 were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
91 and transferred to a polyvinylidene difluoride membrane. After blocking with 5% skim milk
92 in phosphate-buffered saline (PBS), the sheets were probed with rabbit polyclonal anti-BID
93 antibody (Cell Signaling Technology) diluted 1:1000 in PBS. Then, goat anti-rabbit IgG
94 HRP-linked antibody (Cell Signaling Technology) diluted 1:2000 in PBS was added. The
95 bound antibodies were visualized using Immun Star HRP Chemiluminescent Kit (Bio-Rad
96 Laboratories). All visualized bands were documented using Alliance 4.7 (UVItech,
97 Cambridge, UK) and quantified using UVIband software (UVItech).

Comment [BK2]: 1) DAPI staining (nuclei stained) is applied to observe changes in nuclear morphology (condensed and fragmented nuclei).
2) Cell numbers were reduced due to nuclear shrinkage, chromosome condensation and apoptotic bodies.

Comment [BK3]: 1) Why use DMSO as a HONE-1 cells therapy agent?
2) What is the concentration of DMSO used in this study?

Comment [BK4]: 1) Why is the HONE-1 cell treatment for the Bid expression carried out only for 6 hours?
2) Why was the treatment duration not carried out for 24 and 48 hours, as in the DAPI staining?

98 **Data Analyses**

99 Statistical analysis was performed with IBM SPSS Statistics version 26 (IBM, Armonk, NY,
100 USA). Shapiro-Wilk test was performed to analyze the normality of the data. Kruskal-Wallis

101 test followed by *post hoc* Mann-Whitney U test was used to analyze the differences of the
102 number of viable cells between groups at 24 and 48 h. Data was expressed as mean \pm
103 standard deviation (SD) and $p < 0.05$ were considered as statistically significant.

104

105 **Results**

106 **CXRE decreased the amount of viable HONE-1 cells**

107 At 24 and 48 h, the viability of HONE-1 cells in the DMSO-treated group was the highest
108 compared with other groups (Figure 1A, 2A), while the viability of HONE-1 cells in the
109 Doxorubicin-treated group was the lowest (Figure 1B, 2B) as indicated by DAPI staining
110 results. There were 337.83 ± 66.58 and 297.50 ± 81.44 viable HONE-1 cells in the DMSO-
111 treated group at 24 and 48 h, respectively. Meanwhile, there were only 7.00 ± 5.87 and
112 4.83 ± 2.40 viable cells after Doxorubicin treatment for 24 and 48 h, respectively (Figure 3).

113 Upon CXRE addition, the viability of HONE-1 cells was lower compared with that in the
114 DMSO-treated group, implying that CXRE could reduce the viability of HONE-1 cells
115 (Figure 1C-E, 2C-E). The number of viable HONE-1 cells decreased in concentration- and
116 time-dependent manner. At 24 h incubation period, the number of viable cells in $10 \mu\text{g/mL}$
117 CXRE-treated group was not significantly different compared with that in the DMSO-treated
118 group ($p = 0.109$). The number of viable cells in 50 and $250 \mu\text{g/mL}$ CXRE-treated groups were
119 significantly lower compared with that in the DMSO-treated group ($p < 0.05$). Furthermore,
120 the number of viable cells in all CXRE-treated groups were significantly higher compared to
121 that in the Doxorubicin-treated group ($p < 0.05$). Meanwhile, at 48 h incubation period, the
122 number of viable cells in all CXRE-treated groups were significantly lower compared with
123 that in the DMSO-treated group ($p < 0.05$). The number of viable cells in 10 and $50 \mu\text{g/mL}$
124 CXRE-treated groups were significantly higher when compared to the Doxorubicin-treated
125 group ($p < 0.05$), while the number of viable cells in $250 \mu\text{g/mL}$ CXRE-treatment group was

Comment [BK5]: The data had large standard deviation, what were this caused by?

Formatted: Highlight

126 | not significantly different compared to that in the Doxorubicin-treated group ($p=0.872$).

127 CXRE-treated groups had lower number of viable cells at 48 h than those at 24 h (Figure 3).

128 CXRE reduced Bid expression in HONE-1 cells

129 Bid expression levels in 10, 50, and 250 $\mu\text{g/mL}$ CXRE-treated groups were lower compared

130 with that in the DMSO-treated group. CXRE concentration of 250 $\mu\text{g/mL}$ showed greater

131 reduction in Bid expression than the other two concentrations. Bid expression level in

132 HONE-1 cells treated with 50 $\mu\text{g/mL}$ CXRE was slightly higher compared with those treated

133 with 10 $\mu\text{g/mL}$ CXRE. Bid expression levels in the CXRE-treated groups were higher

134 compared with that in Doxorubicin-treated group. No band was observed in the Doxorubicin-

135 treated group (Figure 4).

136

137 Discussion

138 In the present study, CXRE reduced the viability of HONE-1 cells in concentration- and

139 time-dependent manner, which may be caused by apoptosis induction. A previous study

140 reported that combination of Cisplatin, *C. xanthorrhiza* rhizome ethanolic extract and *Ficus*

141 *septica* leaves ethanolic extract enhanced apoptosis of human breast cancer cells, as

142 demonstrated by higher cell death percentage when compared to those that were treated with

143 Cisplatin merely.(24) Active compounds found in *C. xanthorrhiza* rhizomes have also been

144 reported to induce apoptosis of several cancer cells. Xanthorrhizol has been demonstrated to

145 promote apoptosis in human hepatoma (17), promyelocytic leukemia (19), and non-small cell

146 carcinoma cells.(20) Curcumin, another important compounds in rhizomes of *C. xanthorrhiza*

147 and other *Curcuma* species (11,25), has been shown to promote apoptosis in NPC cell lines,

148 such as NPC-TW 076 (26), CNE1 and CNE2 (27), as well as other types of cancer, including

149 prostate cancer (22) and acute myeloid leukemia cells.(28) Interestingly, a study reveals that

Comment [BK6]: "the number of viable cells in 250 $\mu\text{g/mL}$ CXRE-treatment group was not significantly different compared to that in the Doxorubicin-treated group" Why are no bands observed on Doxorubicin-treated group?

150 a combination of xanthorrhizol and curcumin synergistically inhibit cell growth by inducing
151 apoptosis in human breast cancer cells.(29)

152 To confirm whether CXRE promoted apoptosis of HONE-1 cells, the expression
153 levels of Bid were measured. Upon activation of death receptors by apoptotic signals, full
154 length Bid is truncated by cleaved caspase-8 to form truncated Bid (t-Bid). Therefore, Bid
155 activation reduces the amount of Bid and increases the amount of t-Bid in the cell.(23,30) In
156 the present study, Bid expression levels in CXRE-treated HONE-1 cells were lower
157 compared to those in the untreated and sham groups. Thus, it can be concluded that CXRE
158 stimulated Bid activation.

159 CXRE-induced Bid truncation could be related to its active compounds, xanthorrhizol
160 and curcumin. Xanthorrhizol has been demonstrated to decrease Bid expression in several
161 types of cancer cells, such as hepatoma (17), promyelocytic leukemia cells (19), and colon
162 cancer.(31) Curcumin-induced reduction of Bid expression is also reported in several cancer
163 cells, including glioblastoma (32) and B-precursor acute lymphoblastic leukemia cell
164 lines.(33)

165 Xanthorrhizol has been reported to regulate several signaling pathways which
166 modulate apoptosis induction of cancer cells. Xanthorrhizol inhibits proliferation and induces
167 apoptosis of non-small cell carcinoma cells by inhibiting the activation of
168 phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor kappa B (NF- κ B) pathway, which is
169 involved in controlling cell survival.(20) Interestingly, this compound may also be capable in
170 inducing caspase-independent apoptosis via stimulation of reactive oxygen species (ROS)-
171 mediated p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)
172 in human oral squamous cell carcinoma cells.(18)

173 Since the results of the present study showed that CXRE activated Bid, which in turn
174 leads HONE-1 cells to apoptosis, components and phenomena in apoptosis signaling pathway

Comment [BK7]:
"Bid activation reduces the amount of Bid"

- Caspases-8/-10 cleaves Bid to produce tBid, which activates a crosstalk pathway between death receptors and mitochondria.
- tBid translocates to mitochondria where it blocks anti-apoptotic activity of Bcl-2 and Bcl-XL, and activates Bax and Bak.
- This leads to release of cytochrome c and Smac/Diablo and activation of the mitochondrial pathway of apoptosis.

175 both upstream and downstream of Bid, such as DNA fragmentation and $\Delta\Psi_m$ attenuation, as
176 well as the expression level of caspases and apoptogenic factors should be examined.

177

178 **Conclusion**

179 CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1
180 cells. Taken together, CXRE is suggested to have cytotoxic effect towards NPC cells, and it
181 could be a potential anticancer agent for NPC.

182

183 **Author Contribution**

184 DR and FS prepared study concept and design. DR, FS and JH performed processing and
185 acquisition of data. DR, FS, SJA and MSD performed analysis and interpretation of results.
186 DR and JH prepared the draft of the manuscript. FS, SJA and MSD made critical revisions
187 of the manuscript. DR, JH and SJA assisted in administrative, technical, and material
188 support. FS and MSD performed supervision of the study.

189

Comment [BK8]: It is necessary to analyze the effect of CXRE on growth, proliferation, or apoptosis of normal cell

190 **References**

- 191 1. GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020.
192 Nasopharynx, Source: Globocan 2020 [cited 2023 Jan 6]. Available from:
193 <https://gco.iarc.fr/today/data/factsheets/cancers/4-Nasopharynx-fact-sheet.pdf>.
- 194 2. GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020.
195 Indonesia, Source: Globocan 2020 [cited 2023 Jan 6]. Available from:
196 <https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf>.
- 197 3. Teo PML, Chan ATC. Treatment strategy and clinical experience. *Semin Cancer Biol.*
198 2002;12(6):497–504.
- 199 4. Kuhuwael FG, Perkasa MF, Miskad UA, Punagi AQ, Said FA. Comparison of the
200 means of argyrophilic nucleolar organizer region (mAgNOR) pre- and post-therapy in
201 nasopharyngeal carcinoma patients at Wahidin Sudirohusodo General Hospital
202 Makassar. *Indones Biomed J.* 2016;8(2):103–8.
- 203 5. Al Azhar M, Nadliroh S, Prameswari K, Handoko H, Tobing DL, Herawati C. Profile
204 of PD-1 and PD-L1 mRNA expression in peripheral blood of nasopharyngeal
205 carcinoma. *Mol Cell Biomed Sci.* 2020;4(3):121–7.
- 206 6. Vissink A, Jansma J, Spijkervet F, Burlage F, Coppes R. Oral sequelae of head and
207 neck radiotherapy. *Crit Rev Oral Biol Med.* 2003;14(3):199–212.
- 208 7. Sandra F. Targeting ameloblastoma into apoptosis. *Indones Biomed J.* 2018;10(1):35–9.
- 209 8. Novilla A, Mustofa M, Astuti I, Jumina J, Suwito H. Cytotoxic activity of methoxy-
210 4’-amino chalcone derivatives against leukemia cell lines. *Mol Cell Biomed Sci.*
211 2019;3(1):34–41.
- 212 9. Ngadino, Setiawan, Koerniasari, Ernawati, Sudjarwo S. Evaluation of
213 antimycobacterial activity of *Curcuma xanthorrhiza* ethanolic extract against
214 *Mycobacterium tuberculosis* H37Rv in vitro. *Vet World.* 2018;11(3):368–72.
- 215 10. Yogiara, Mordukhova EA, Kim D, Kim WG, Hwang JK, Pan JG. The food-grade
216 antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in *Escherichia coli*.
217 *Bioorg Med Chem Lett.* 2020;30(24):127651.
- 218 11. Jantan I, Saputri FC, Qaisar MN, Buang F. Correlation between chemical composition
219 of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human
220 low-density lipoprotein oxidation. *Evid Based Complement Alternat Med.*
221 2012;2012:438356.
- 222 12. Devaraj S, Ismail S, Ramanathan S, Yam MF. Investigation of antioxidant and
223 hepatoprotective activity of standardized *Curcuma xanthorrhiza* rhizome in carbon

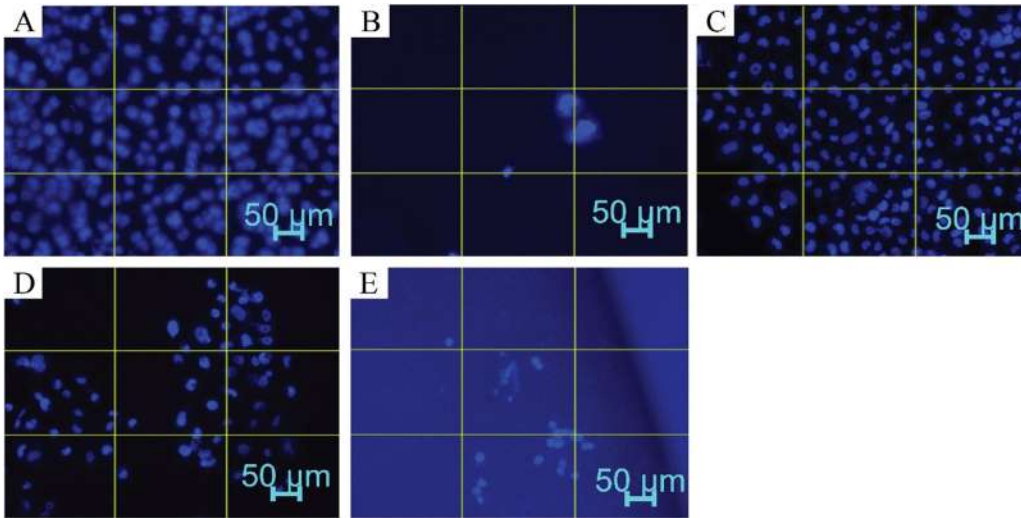
- 224 tetrachloride-induced hepatic damaged rats. *ScientificWorldJournal*.
225 2014;2014:353128.
- 226 13. Kim MB, Kim C, Song Y, Hwang JK. Antihyperglycemic and anti-inflammatory
227 effects of standardized *Curcuma xanthorrhiza* Roxb. extract and its active compound
228 xanthorrhizol in high-fat diet-induced obese mice. *Evid Based Complement Alternat*
229 *Med*. 2014;2014:205915.
- 230 14. Kim S, Kook KE, Kim C, Hwang JK. Inhibitory effects of *Curcuma xanthorrhiza*
231 supercritical extract and xanthorrhizol on LPS-induced inflammation in HGF-1 cells
232 and RANKL-induced osteoclastogenesis in RAW264.7 cells. *J Microbiol Biotechnol*.
233 2018;28(8):1270–81.
- 234 15. Park JH, Park KK, Kim MJ, Hwang JK, Park SK, Chung WY. Cancer chemoprotective
235 effects of *Curcuma xanthorrhiza*. *Phytother Res*. 2008;22(5):695–8.
- 236 16. Zhang CM, Fan PH, Li M, Lou HX. Two new sesquiterpenoids from the rhizomes of
237 *Curcuma xanthorrhiza*. *Helv Chim Acta*. 2014 Sep;97(9):1295–300.
- 238 17. Tee TT, Cheah YH, Meenakshii N, Mohd Sharom MY, Azimahtol Hawariah LP.
239 Xanthorrhizol induced DNA fragmentation in HepG2 cells involving Bcl-2 family
240 proteins. *Biochem Biophys Res Commun*. 2012;420(4):834–8.
- 241 18. Kim JY, An JM, Chung WY, Park KK, Hwang JK, Kim DS, et al. Xanthorrhizol
242 induces apoptosis through ROS-mediated MAPK activation in human oral squamous
243 cell carcinoma cells and inhibits DMBA-induced oral carcinogenesis in hamsters.
244 *Phytother Res*. 2013;27(4):493–8.
- 245 19. Kim HJ, Chung WY, Hwang JK, Park KK. Xanthorrhizol induces apoptotic cell death
246 through molecular cross talks between mitochondria-dependent and death receptor-
247 mediated signaling in human promyelocytic leukemia cells. *Cancer Prev Res*.
248 2013;18:41–7.
- 249 20. Cai Y, Sheng Z, Wang J. Xanthorrhizol inhibits non-small cell carcinoma (A549) cell
250 growth and promotes apoptosis through modulation of PI3K/AKT and NF- κ B signaling
251 pathway. *Environ Toxicol*. 2022;37(1):120–30.
- 252 21. Luo P, Cheng Y, Yin Z, Li C, Xu J, Gu Q. Monomeric and dimeric cytotoxic
253 guaianolide-type sesquiterpenoids from the aerial parts of *Chrysanthemum indicum*. *J*
254 *Nat Prod*. 2019;82(2):349–57.
- 255 22. Pan L, Sha J, Lin W, Wang Y, Bian T. Curcumin inhibits prostate cancer progression
256 by regulating the miR-30a-5p/PCLAF axis. *Exp Ther Med*. 2021;22(3):969.
- 257 23. Lin HF, Hsieh MJ, Hsi YT, Lo YS, Chuang YC, Chen MK, et al. Celastrol-induced
258 apoptosis in human nasopharyngeal carcinoma is associated with the activation of the
259 death receptor and the mitochondrial pathway. *Oncol Lett*. 2017;14(2):1683–90.

- 260 24. Hidayati DN, Jenie RI, Meiyanto E. Combination of curcuma (*Curcuma xanthorrhiza*
261 Roxb) rhizome ethanolic extract and awar-awar (*Ficus septica* Burm.F) leaves ethanolic
262 extract increases Cisplatin cytotoxicity on T47D breast cancer cells through cell cycle
263 modulation. *Indones J Cancer Chemoprevent*. 2017;8(3):114–8.
- 264 25. Rahmat E, Lee J, Kang Y. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.):
265 Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. *Evid*
266 *Based Complement Alternat Med*. 2021;2021:9960813.
- 267 26. Kuo C, Wu S, Ip S, Wu P, Yu C, Yang J, et al. Apoptotic death in curcumin-treated
268 NPC-TW 076 human nasopharyngeal carcinoma cells is mediated through the ROS,
269 mitochondrial depolarization and caspase-3-dependent signaling responses. *Int J Oncol*.
270 2011;39(2):319–28.
- 271 27. Feng S, Wang Y, Zhang R, Yang G, Liang Z, Wang Z, et al. Curcumin exerts its
272 antitumor activity through regulation of miR-7/Skp2/p21 in nasopharyngeal carcinoma
273 cells. *Onco Targets Ther*. 2017;10:2377–88.
- 274 28. Zhou H, Ning Y, Zeng G, Zhou C, Ding X. Curcumin promotes cell cycle arrest and
275 apoptosis of acute myeloid leukemia cells by inactivating AKT. *Oncol Rep*. 2021 Feb
276 2;45(4):11.
- 277 29. Cheah Y, Nordin F, Sarip R, Tee T, Azimahtol H, Sirat HM, et al. Combined
278 xanthorrhizol-curcumin exhibits synergistic growth inhibitory activity via apoptosis
279 induction in human breast cancer cells MDA-MB-231. *Cancer Cell Int*. 2009;9(1):1.
- 280 30. Sandra F, Sidharta MA. Caffeic acid induced apoptosis in MG63 osteosarcoma cells
281 through activation of caspases. *Mol Cell Biomed Sci*. 2017;1(1):28–33.
- 282 31. Kang YJ, Park KK, Chung WY, Hwang JK, Lee SK. Xanthorrhizol, a natural
283 sesquiterpenoid, induces apoptosis and growth arrest in HCT116 human colon cancer
284 cells. *J Pharmacol Sci*. 2009;111(3):276–84.
- 285 32. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and
286 activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG
287 cells. *Neurochem Res*. 2007;32(12):2103–13.
- 288 33. Kuttikrishnan S, Siveen KS, Prabhu KS, Khan AQ, Ahmed EI, Akhtar S, et al.
289 Curcumin induces apoptotic cell death via inhibition of PI3-kinase/AKT pathway in B-
290 precursor acute lymphoblastic leukemia. *Front Oncol*. 2019;9:484.

291

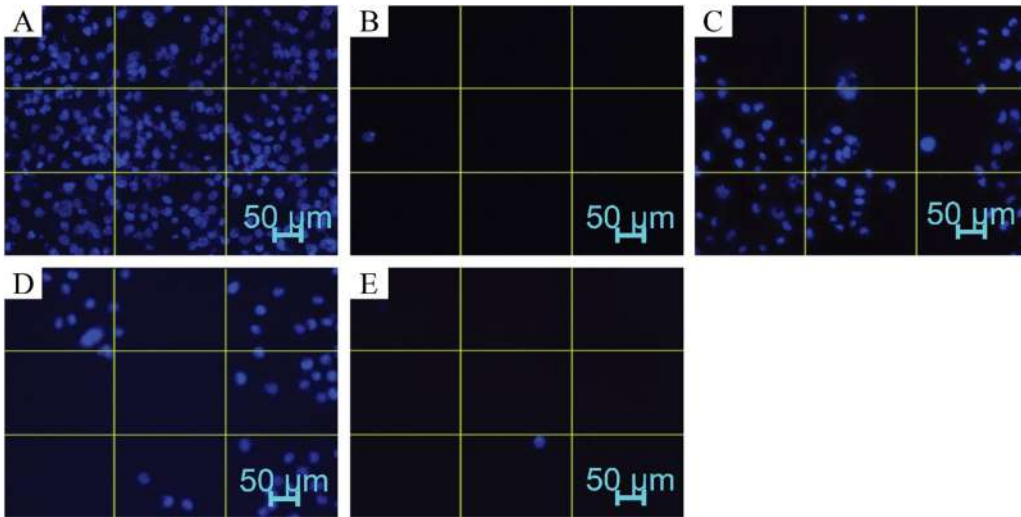
292

293 **Figures**



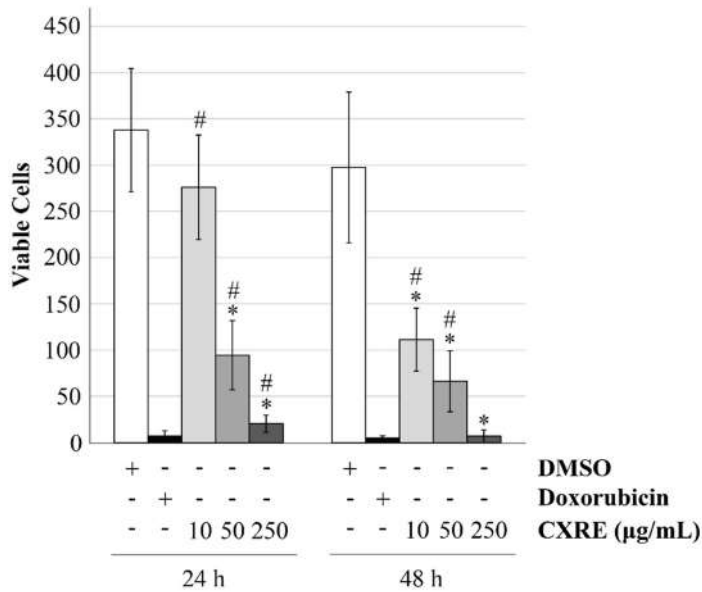
294
295 **Figure 1. CXRE reduced the viability of HONE-1 cells after 24 h.** HONE-1 cells were seeded, starved for 12
296 h, and treated with DMSO, 3 µM Doxorubicin, or various concentrations of CXRE for 24 h. Cell nuclei were
297 stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B:
298 Doxorubicin; C: 10 µg/mL CXRE; D: 50 µg/mL CXRE; E: 250 µg/mL CXRE.

Comment [BK9]: Why are HONE-1 cells starved for 12 hours prior to treatment?



299
300 **Figure 2. CXRE reduced the viability of HONE-1 cells after 48 h.** HONE-1 cells were seeded, starved for 12
301 h, and treated with DMSO, 3 µM Doxorubicin, or various concentrations of CXRE for 48 h. Cell nuclei were
302 stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B:
303 Doxorubicin; C: 10 µg/mL CXRE; D: 50 µg/mL CXRE; E: 250 µg/mL CXRE.

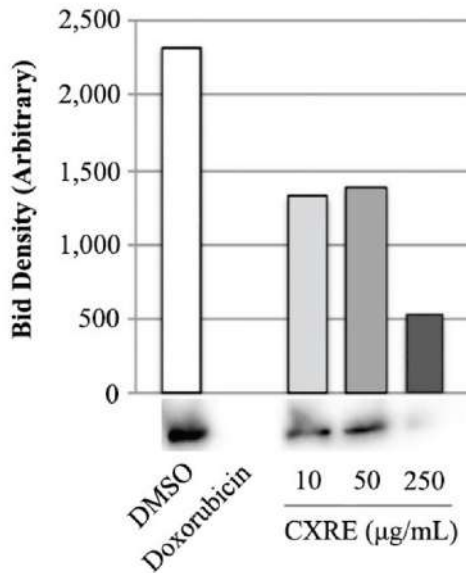
Comment [BK10]: Why are HONE-1 cells starved for 12 hours prior to treatment?



304

305 **Figure 3. CXRE decreased the number of viable HONE-1 cells in concentration- and time-dependent**
 306 **manner.** HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 µM Doxorubicin, or various
 307 concentrations of CXRE for 24 and 48 h. Cell nuclei were stained with DAPI. The number of viable cells in
 308 each slide was evaluated by two independent observers as described in Methods. The data were expressed as
 309 mean ± SD (n=3). * $p < 0.05$ vs. DMSO-treated group; # $p < 0.05$ vs. Doxorubicin-treated group.

Comment [BK11]: Why are HONE-1 cells starved for 12 hours prior to treatment?



310

311 **Figure 4. CXRE diminished Bid expression in HONE-1 cells.** HONE-1 cells were seeded, starved for 12 h,
 312 and treated with DMSO, 3 µM Doxorubicin, or 10, 50, or 250 µg/mL CXRE for 6 h as indicated in the panel.
 313 Cells were collected, lysed, and further processed to obtain cell lysate for Western blot analysis as described
 314 in Methods.

Comment [BK12]: Why are HONE-1 cells starved for 12 hours prior to treatment?



Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023009 Editor Decision Round 1 - Revisions Required

Ferry Sandra <ferry@trisakti.ac.id>

Thu, Jan 19, 2023 at 9:59 AM

To: Secretariat of InaBJ <secretariatnabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Thank you for sharing the review outcomes. Attached is the revised manuscript M2023009 titled "*Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid". I have made the necessary revisions based on the feedback received.

Thank you.

Regards,

Ferry Sandra

[Quoted text hidden]

--

Ferry Sandra, D.D.S., Ph.D.

Head of Medical Research Center

Universitas Trisakti

**Round 1 Revision from Author.docx**

611K

1 ***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal**
2 **Cancer Cells Through Bid**

3
4 **Abstract**

5 **Background:** *Curcuma xanthorrhiza* rhizomes have been demonstrated to have anticancer
6 properties toward various types of cancer cells. The effect of *C. xanthorrhiza* rhizome extract
7 (CXRE) on nasopharyngeal cancer (NPC) cells, including HONE-1 cell line has not been
8 elucidated yet. Therefore, the effect of CXRE on the apoptosis of HONE-1 cells and its
9 possible underlying mechanism are necessary to be explored.

10 **Methods:** *C. xanthorrhiza* rhizomes were minced, dried, extracted with distilled ethanol,
11 filtered, and evaporated to produce CXRE. HONE-1 cells were seeded, starved, and treated
12 with dimethyl sulfoxide (DMSO), Doxorubicin, or various concentrations of CXRE. Treated
13 HONE-1 cells were stained with 4',6'-diamidino-2-phenylindole (DAPI) and the number of
14 viable cells was counted. HONE-1 cells were also collected, lysed, and further processed for
15 immunoblotting analysis to measure Bid activity.

16 **Results:** The number of viable HONE-1 cells decreased in concentration- and time-
17 dependent manner. The number of viable cells in 50 and 250 µg/mL CXRE-treated groups
18 were significantly lower compared with that in the DMSO-treated group after 24 h. At 48 h
19 incubation period, the number of viable cells in 10, 50 and 250 µg/mL CXRE-treated groups
20 were significantly lower compared with that in the DMSO-treated group. The number of
21 viable cells in 250 µg/mL CXRE-treatment group was not significantly different compared
22 with that in the Doxorubicin-treated group after 48 h. Bid expression levels in CXRE-treated
23 groups were lower compared with that in the DMSO-treated group.

24 **Conclusion:** CXRE could induce apoptosis via Bid activation, hence reducing the viability of
25 HONE-1 cells.

26 **Keywords:** *Curcuma xanthorrhiza*, nasopharyngeal cancer, HONE-1 cells, apoptosis, Bid

27

28 **Introduction**

29 There are 133,354 new nasopharyngeal cancer (NPC) cases and 80,008 deaths worldwide due
30 to this type of cancer in 2020.(1) NPC is considered as the fifth most common cancer in
31 Indonesia after breast, cervix uteri, lung, and liver cancers, with 19,943 new cases and 13,399
32 deaths.(2) NPC is generally treated with radiotherapy, while combination of radiotherapy and
33 chemotherapy is used to treat advance-stage NPC.(3–5) The standard treatments have been
34 reported to cause numerous adverse effects, some of which are permanent.(6) Advancement
35 in cancer treatment and the discovery of novel anticancer agents is constantly growing. One
36 of the main focuses of the recent cancer research is the development of anticancer agents
37 from natural substances or their derivatives since they are believed to have a potential to
38 inhibit cancer development and progression without affecting normal cells.(7,8)

39 *Curcuma xanthorrhiza* D.Dietr. is a medicinal plant that belongs to Zingiberaceae
40 family. The rhizome of this plant has been reported to have numerous pharmacological
41 activities, such as antibacterial (9,10), antioxidant (11,12), anti-inflammatory (13,14) and
42 anticancer properties.(15) These properties are due to the presence of natural compounds,
43 which are dominated by curcuminoids and terpenoids.(16) Xanthorrhizol, the main
44 compound of *C. xanthorrhiza* that distinguishes this species with other *Curcuma* species, has
45 been demonstrated to show anticancer activities on several types of human cancer cells,
46 including hepatoma (17), oral squamous cell carcinoma (18), promyelocytic leukemia (19),
47 and non-small cell carcinoma.(20)

48 HONE-1, an NPC cell line, is often used to investigate the cytotoxic effect of
49 compounds obtained from a medicinal plant.(21) This cell line has also been used in research
50 that assesses cytotoxicity of extract obtained from *Curcuma* sp. However, the effect of *C.*

51 *xanthorrhiza* rhizome extract (CXRE) on NPC cells, including HONE-1 cell line has not been
52 elucidated yet.

53 The cytotoxicity of compounds found in *C. xanthorrhiza* rhizomes on different types
54 of cancer cells may be related to apoptosis.(17–20,22) One of the signaling pathway that
55 could be activated by these compounds to initiate apoptosis is intrinsic apoptotic pathway,
56 which involves activation of B-cell lymphoma (Bcl)-2 homology 3-interacting domain death
57 agonist (Bid). Apoptotic stimuli induce Bid truncation to form truncated Bid (t-Bid). t-Bid
58 increases mitochondrial membrane permeability, which in turn causes the release of
59 apoptogenic factors from mitochondria to cytoplasm. These apoptogenic factors promote the
60 activation of effector caspases that play a critical role in executing cell death.(19,23) Since *C.*
61 *xanthorrhiza* has been reported to show anticancer potential in various types of cancer, the
62 effect of CXRE on the apoptosis of HONE-1 NPC cells and its possible underlying
63 mechanism are necessary to be explored.

64

65 **Methods**

66 **Plant Sample Collection and Extraction**

67 *C. xanthorrhiza* rhizome samples were collected from Bogor, Indonesia. The rhizomes were
68 identified and extracted in PT. Aretha Medika Utama, Bandung, Indonesia. Briefly, *C.*
69 *xanthorrhiza* rhizomes were minced, dried, extracted with distilled 70% ethanol for 24 h at
70 room temperature, and evaporated. The resulting CXRE was then stored at -20°C.

71

72 **HONE-1 Cell Culture**

73 HONE-1 cells were cultured in RPMI 1640 without L-Gln (Gibco, Grand Island, NY, USA)
74 supplemented with 10% fetal bovine serum (Gibco) and 1% Penicillin-Streptomycin-
75 Amphotericin B (Gibco). The cells were maintained in an incubator at 37°C with 5% CO₂.

76

77 **4',6'-diamidino-2-phenylindole (DAPI) Staining**

78 HONE-1 cells were seeded onto coverslips, treated with/without dimethyl sulfoxide (DMSO),
79 3 µM Doxorubicin (Dankos Farma, Jakarta, Indonesia), or 10, 50, or 250 µg/mL CXRE for
80 24 or 48 h. CXRE-treated HONE-1 cells were fixed with 70% ethanol for 3 minutes and
81 washed in PBS. HONE-1 cells were then fixed with 0.1% Triton X-100 for 1 minute and
82 stained by applying 1:100 diluted DAPI (Sigma-Aldrich, St. Louis, USA). HONE-1 cells
83 were evaluated and documented under a fluorescence microscope in three replicates. In each
84 slide, viable cell number was counted by using grids by two independent observers.

85

86 **Immunoblotting**

87 HONE-1 cells were seeded and treated with/without DMSO, 3 µM Doxorubicin, or 10, 50, or
88 250 µg/mL CXRE for 6 h. Treated HONE-1 cells were lysed with a lysis buffer containing
89 10X radio-immunoprecipitation assay (RIPA) buffer (Abcam, Cambridge, UK) and
90 phenylmethanesulfonyl fluoride (Sigma-Aldrich). Twenty µL lysates were sodium dodecyl
91 sulfate-polyacrylamide gel electrophoresed, followed by the transfer onto the polyvinylidene
92 difluoride membrane. Blocking was performed with 5% skim milk, then the sheets were
93 probed with rabbit polyclonal anti-BID antibody (Cell Signaling Technology) diluted 1:1000
94 in phosphate-buffered saline (PBS). Then, goat anti-rabbit IgG HRP-linked antibody (Cell
95 Signaling Technology) diluted 1:2000 in PBS was added. Immun Star HRP

96 Chemiluminescent Kit (Bio-Rad Laboratories) was used to visualize the bands while Alliance
97 4.7 (UVItech, Cambridge, UK) was used to capture and quantify the bands.

98

99 **Data Analyses**

100 Statistical analysis was performed with IBM SPSS Statistics version 26 (IBM, Armonk, NY,
101 USA). Shapiro-Wilk test was performed to analyze the normality of the data. To analyze the
102 differences of the number of viable cells between groups at 24 and 48 h, Kruskal-Wallis test
103 followed by *post hoc* Mann-Whitney U test were used.

104

105 **Results**

106 **CXRE decreased the amount of viable HONE-1 cells**

107 At 24 and 48 h, the viability of HONE-1 cells in the DMSO-treated group was the highest
108 compared with other groups (Figure 1A, 2A), while the viability of HONE-1 cells in the
109 Doxorubicin-treated group was the lowest (Figure 1B, 2B) as indicated by DAPI staining
110 results. There were 337.83 ± 66.58 and 297.50 ± 81.44 viable HONE-1 cells in the DMSO-
111 treated group at 24 and 48 h, respectively. Meanwhile, there were only 7.00 ± 5.87 and
112 4.83 ± 2.40 viable cells after Doxorubicin treatment for 24 and 48 h, respectively (Figure 3).
113 Upon CXRE addition, the viability of HONE-1 cells was lower compared with that in the
114 DMSO-treated group, implying that CXRE could reduce the viability of HONE-1 cells
115 (Figure 1C-E, 2C-E). The number of viable HONE-1 cells decreased in concentration- and
116 time-dependent manner. The number of viable cells in CXRE-treated groups were
117 significantly lower compared with that in DMSO-treated group ($p < 0.05$), except for 10
118 $\mu\text{g/mL}$ CXRE-treated group at 24 h ($p = 0.109$). However, the number of viable cells in
119 CXRE-treated groups were significantly higher compared with that in Doxorubicin-treated

120 group ($p < 0.05$), except for 250 $\mu\text{g}/\text{mL}$ CXRE-treatment group at 48 h ($p = 0.872$). CXRE-
121 treated groups had lower number of viable cells at 48 h than those at 24 h (Figure 3).

122

123 **CXRE reduced Bid expression in HONE-1 cells**

124 Bid expression levels in 10, 50, and 250 $\mu\text{g}/\text{mL}$ CXRE-treated groups were lower compared
125 with that in the DMSO-treated group. CXRE concentration of 250 $\mu\text{g}/\text{mL}$ showed greater
126 reduction in Bid expression than the other two concentrations. Bid expression level in
127 HONE-1 cells treated with 50 $\mu\text{g}/\text{mL}$ CXRE was slightly higher compared with those treated
128 with 10 $\mu\text{g}/\text{mL}$ CXRE. Bid expression levels in the CXRE-treated groups were higher
129 compared with that in Doxorubicin-treated group. No band was observed in the Doxorubicin-
130 treated group (Figure 4).

131

132 **Discussion**

133 In the present study, CXRE reduced the viability of HONE-1 cells in concentration-
134 and time-dependent manner, which may be caused by apoptosis induction. A previous study
135 reported that combination of Cisplatin, *C. xanthorrhiza* rhizome ethanolic extract and *Ficus*
136 *septica* leaves ethanolic extract enhanced apoptosis of human breast cancer cells, as
137 demonstrated by higher cell death percentage when compared with those that were treated
138 with Cisplatin merely.(24) Active compounds found in *C. xanthorrhiza* rhizomes have also
139 been reported to induce apoptosis of several cancer cells. Xanthorrhizol has been
140 demonstrated to promote apoptosis in human hepatoma (17), promyelocytic leukemia (19),
141 and non-small cell carcinoma cells.(20) Curcumin, another important compounds in rhizomes
142 of *C. xanthorrhiza* and other *Curcuma* species (11,25), has been shown to promote apoptosis
143 in NPC cell lines, such as NPC-TW 076 (26), CNE1 and CNE2 (27), as well as other types of
144 cancer, including prostate cancer (22) and acute myeloid leukemia cells.(28) Interestingly, a

145 study reveals that a combination of xanthorrhizol and curcumin synergistically inhibit cell
146 growth by inducing apoptosis in human breast cancer cells.(29)

147 To confirm whether CXRE promoted apoptosis of HONE-1 cells, the expression
148 levels of Bid were measured. Upon activation of death receptors by apoptotic signals, full
149 length Bid is truncated by cleaved caspase-8 to form truncated Bid (t-Bid), which
150 interconnects intrinsic and extrinsic apoptotic pathways. Hence, upon activation, the amount
151 of Bid is decreased while the amount of t-Bid is increased in the cell. tBid then translocates to
152 mitochondria where it blocks anti-apoptotic activity of Bcl-extra-large (Bcl-X_L) and Bcl-2,
153 and activates proapoptotic Bcl-2-associated X protein (Bax) and Bcl-2 homologous
154 antagonist killer (Bak). This leads to the release of second mitochondria-derived activator of
155 caspase (Smac)/direct inhibitor of apoptosis protein-binding protein with low pI (DIABLO)
156 and cytochrome *c*, which play critical roles in executing cell death.(19,23,30) In the present
157 study, Bid expression levels in CXRE-treated HONE-1 cells were lower compared with those
158 in the DMSO-treated group. Thus, it can be concluded that CXRE stimulated Bid activation.

159 CXRE-induced Bid truncation could be related to its active compounds, xanthorrhizol
160 and curcumin. Xanthorrhizol has been demonstrated to decrease Bid expression in several
161 types of cancer cells, such as hepatoma (17), promyelocytic leukemia cells (19), and colon
162 cancer.(31) Curcumin-induced reduction of Bid expression is also reported in several cancer
163 cells, including glioblastoma (32) and B-precursor acute lymphoblastic leukemia cell
164 lines.(33)

165 Xanthorrhizol has been reported to regulate several signaling pathways which
166 modulate apoptosis induction of cancer cells. Xanthorrhizol inhibits proliferation and induces
167 apoptosis of non-small cell carcinoma cells by inhibiting the activation of
168 phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor kappa B (NF-κB) pathway, which is
169 involved in controlling cell survival.(20) Interestingly, this compound may also be capable in

170 inducing caspase-independent apoptosis via stimulation of reactive oxygen species (ROS)-
171 mediated p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)
172 in human oral squamous cell carcinoma cells.(18)

173 Since the results of the present study showed that CXRE activated Bid, which in turn
174 leads HONE-1 cells to apoptosis, components and phenomena in apoptosis signaling pathway
175 both upstream and downstream of Bid, such as DNA fragmentation and $\Delta\Psi_m$ attenuation, as
176 well as the expression level of caspases and apoptogenic factors should be examined.

177

178 **Conclusion**

179 CXRE could induce apoptosis via Bid activation, hence reducing the viability of HONE-1
180 cells. Taken together, CXRE is suggested to have cytotoxic effect towards NPC cells, and it
181 could be a potential anticancer agent for NPC.

182

183 **Author Contribution**

184 DR and FS prepared study concept and design. DR, FS and JH performed processing and
185 acquisition of data. DR, FS, SJAI and MSD performed analysis and interpretation of results.
186 DR and JH prepared the draft of the manuscript. FS, SJAI and MSD made critical revisions
187 of the manuscript. DR, JH and SJAI assisted in administrative, technical, and material
188 support. FS and MSD performed supervision of the study.

189

190 **References**

- 191 1. GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020.
 192 Nasopharynx, Source: Globocan 2020 [cited 2023 Jan 6]. Available from:
 193 <https://gco.iarc.fr/today/data/factsheets/cancers/4-Nasopharynx-fact-sheet.pdf>.
- 194 2. GLOBOCAN [Internet]. Lyon: International Agency for Research on Cancer; ©2020.
 195 Indonesia, Source: Globocan 2020 [cited 2023 Jan 6]. Available from:
 196 <https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf>.
- 197 3. Teo PML, Chan ATC. Treatment strategy and clinical experience. *Semin Cancer Biol.*
 198 2002;12(6):497–504.
- 199 4. Kuhuwael FG, Perkasa MF, Miskad UA, Punagi AQ, Said FA. Comparison of the
 200 means of argyrophilic nucleolar organizer region (mAgNOR) pre- and post-therapy in
 201 nasopharyngeal carcinoma patients at Wahidin Sudirohusodo General Hospital
 202 Makassar. *Indones Biomed J.* 2016;8(2):103–8.
- 203 5. Al Azhar M, Nadliroh S, Prameswari K, Handoko H, Tobing DL, Herawati C. Profile
 204 of PD-1 and PD-L1 mRNA expression in peripheral blood of nasopharyngeal
 205 carcinoma. *Mol Cell Biomed Sci.* 2020;4(3):121–7.
- 206 6. Vissink A, Jansma J, Spijkervet F, Burlage F, Coppes R. Oral sequelae of head and
 207 neck radiotherapy. *Crit Rev Oral Biol Med.* 2003;14(3):199–212.
- 208 7. Sandra F. Targeting ameloblastoma into apoptosis. *Indones Biomed J.* 2018;10(1):35–9.
- 209 8. Novilla A, Mustofa M, Astuti I, Jumina J, Suwito H. Cytotoxic activity of methoxy-
 210 4' amino chalcone derivatives against leukemia cell lines. *Mol Cell Biomed Sci.*
 211 2019;3(1):34–41.
- 212 9. Ngadino, Setiawan, Koerniasari, Ernawati, Sudjarwo S. Evaluation of
 213 antimycobacterial activity of *Curcuma xanthorrhiza* ethanolic extract against
 214 *Mycobacterium tuberculosis* H37Rv in vitro. *Vet World.* 2018;11(3):368–72.
- 215 10. Yogiara, Mordukhova EA, Kim D, Kim WG, Hwang JK, Pan JG. The food-grade
 216 antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in *Escherichia coli*.
 217 *Bioorg Med Chem Lett.* 2020;30(24):127651.
- 218 11. Jantan I, Saputri FC, Qaisar MN, Buang F. Correlation between chemical composition
 219 of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human
 220 low-density lipoprotein oxidation. *Evid Based Complement Alternat Med.*
 221 2012;2012:438356.
- 222 12. Devaraj S, Ismail S, Ramanathan S, Yam MF. Investigation of antioxidant and
 223 hepatoprotective activity of standardized *Curcuma xanthorrhiza* rhizome in carbon

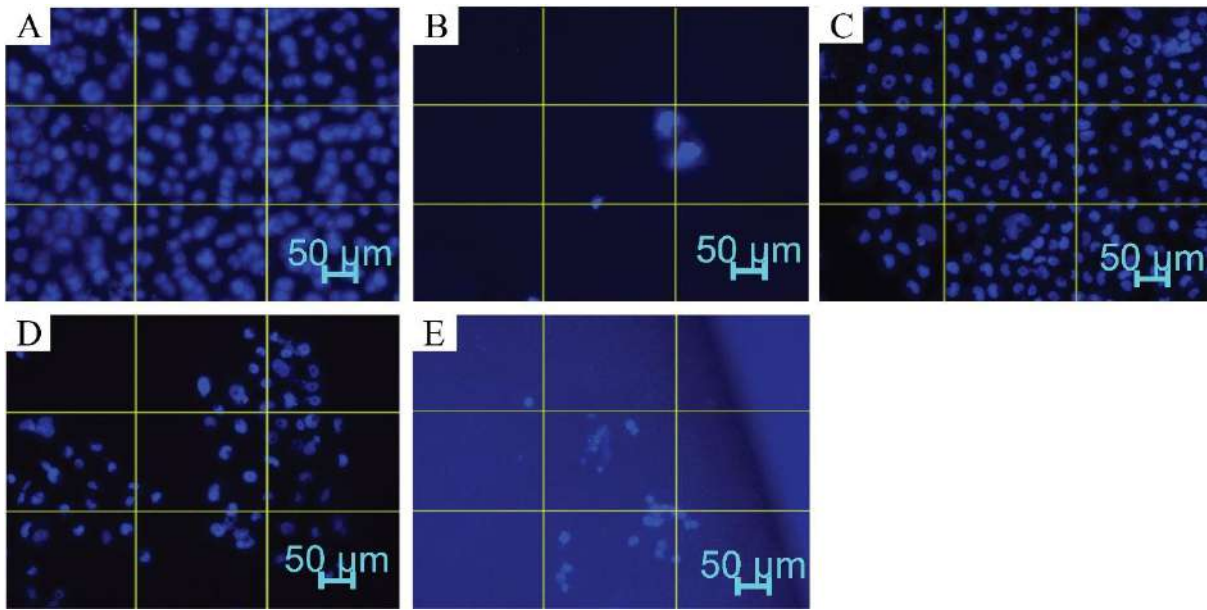
- 224 tetrachloride-induced hepatic damaged rats. *ScientificWorldJournal*.
225 2014;2014:353128.
- 226 13. Kim MB, Kim C, Song Y, Hwang JK. Antihyperglycemic and anti-inflammatory
227 effects of standardized *Curcuma xanthorrhiza* Roxb. extract and its active compound
228 xanthorrhizol in high-fat diet-induced obese mice. *Evid Based Complement Alternat*
229 *Med*. 2014;2014:205915.
- 230 14. Kim S, Kook KE, Kim C, Hwang JK. Inhibitory effects of *Curcuma xanthorrhiza*
231 supercritical extract and xanthorrhizol on LPS-induced inflammation in HGF-1 cells
232 and RANKL-induced osteoclastogenesis in RAW264.7 cells. *J Microbiol Biotechnol*.
233 2018;28(8):1270–81.
- 234 15. Park JH, Park KK, Kim MJ, Hwang JK, Park SK, Chung WY. Cancer chemoprotective
235 effects of *Curcuma xanthorrhiza*. *Phytother Res*. 2008;22(5):695–8.
- 236 16. Zhang CM, Fan PH, Li M, Lou HX. Two new sesquiterpenoids from the rhizomes of
237 *Curcuma xanthorrhiza*. *Helv Chim Acta*. 2014 Sep;97(9):1295–300.
- 238 17. Tee TT, Cheah YH, Meenakshii N, Mohd Sharom MY, Azimahtol Hawariah LP.
239 Xanthorrhizol induced DNA fragmentation in HepG2 cells involving Bcl-2 family
240 proteins. *Biochem Biophys Res Commun*. 2012;420(4):834–8.
- 241 18. Kim JY, An JM, Chung WY, Park KK, Hwang JK, Kim DS, et al. Xanthorrhizol
242 induces apoptosis through ROS-mediated MAPK activation in human oral squamous
243 cell carcinoma cells and inhibits DMBA-induced oral carcinogenesis in hamsters.
244 *Phytother Res*. 2013;27(4):493–8.
- 245 19. Kim HJ, Chung WY, Hwang JK, Park KK. Xanthorrhizol induces apoptotic cell death
246 through molecular cross talks between mitochondria-dependent and death receptor-
247 mediated signaling in human promyelocytic leukemia cells. *Cancer Prev Res*.
248 2013;18:41–7.
- 249 20. Cai Y, Sheng Z, Wang J. Xanthorrhizol inhibits non-small cell carcinoma (A549) cell
250 growth and promotes apoptosis through modulation of PI3K/AKT and NF- κ B signaling
251 pathway. *Environ Toxicol*. 2022;37(1):120–30.
- 252 21. Luo P, Cheng Y, Yin Z, Li C, Xu J, Gu Q. Monomeric and dimeric cytotoxic
253 guaianolide-type sesquiterpenoids from the aerial parts of *Chrysanthemum indicum*. *J*
254 *Nat Prod*. 2019;82(2):349–57.
- 255 22. Pan L, Sha J, Lin W, Wang Y, Bian T. Curcumin inhibits prostate cancer progression
256 by regulating the miR-30a-5p/PCLAF axis. *Exp Ther Med*. 2021;22(3):969.
- 257 23. Lin HF, Hsieh MJ, Hsi YT, Lo YS, Chuang YC, Chen MK, et al. Celastrol-induced
258 apoptosis in human nasopharyngeal carcinoma is associated with the activation of the
259 death receptor and the mitochondrial pathway. *Oncol Lett*. 2017;14(2):1683–90.

- 260 24. Hidayati DN, Jenie RI, Meiyanto E. Combination of curcuma (*Curcuma xanthorrhiza*
 261 Roxb) rhizome ethanolic extract and awar-awar (*Ficus septica* Burm.F) leaves ethanolic
 262 extract increases Cisplatin cytotoxicity on T47D breast cancer cells through cell cycle
 263 modulation. *Indones J Cancer Chemoprevent.* 2017;8(3):114–8.
- 264 25. Rahmat E, Lee J, Kang Y. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.):
 265 Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. *Evid*
 266 *Based Complement Alternat Med.* 2021;2021:9960813.
- 267 26. Kuo C, Wu S, Ip S, Wu P, Yu C, Yang J, et al. Apoptotic death in curcumin-treated
 268 NPC-TW 076 human nasopharyngeal carcinoma cells is mediated through the ROS,
 269 mitochondrial depolarization and caspase-3-dependent signaling responses. *Int J Oncol.*
 270 2011;39(2):319–28.
- 271 27. Feng S, Wang Y, Zhang R, Yang G, Liang Z, Wang Z, et al. Curcumin exerts its
 272 antitumor activity through regulation of miR-7/Skp2/p21 in nasopharyngeal carcinoma
 273 cells. *Onco Targets Ther.* 2017;10:2377–88.
- 274 28. Zhou H, Ning Y, Zeng G, Zhou C, Ding X. Curcumin promotes cell cycle arrest and
 275 apoptosis of acute myeloid leukemia cells by inactivating AKT. *Oncol Rep.* 2021 Feb
 276 2;45(4):11.
- 277 29. Cheah Y, Nordin F, Sarip R, Tee T, Azimahtol H, Sirat HM, et al. Combined
 278 xanthorrhizol-curcumin exhibits synergistic growth inhibitory activity via apoptosis
 279 induction in human breast cancer cells MDA-MB-231. *Cancer Cell Int.* 2009;9(1):1.
- 280 30. Sandra F, Sidharta MA. Caffeic acid induced apoptosis in MG63 osteosarcoma cells
 281 through activation of caspases. *Mol Cell Biomed Sci.* 2017;1(1):28–33.
- 282 31. Kang YJ, Park KK, Chung WY, Hwang JK, Lee SK. Xanthorrhizol, a natural
 283 sesquiterpenoid, induces apoptosis and growth arrest in HCT116 human colon cancer
 284 cells. *J Pharmacol Sci.* 2009;111(3):276–84.
- 285 32. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and
 286 activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG
 287 cells. *Neurochem Res.* 2007;32(12):2103–13.
- 288 33. Kuttikrishnan S, Siveen KS, Prabhu KS, Khan AQ, Ahmed EI, Akhtar S, et al.
 289 Curcumin induces apoptotic cell death via inhibition of PI3-kinase/AKT pathway in B-
 290 precursor acute lymphoblastic leukemia. *Front Oncol.* 2019;9:484.

291

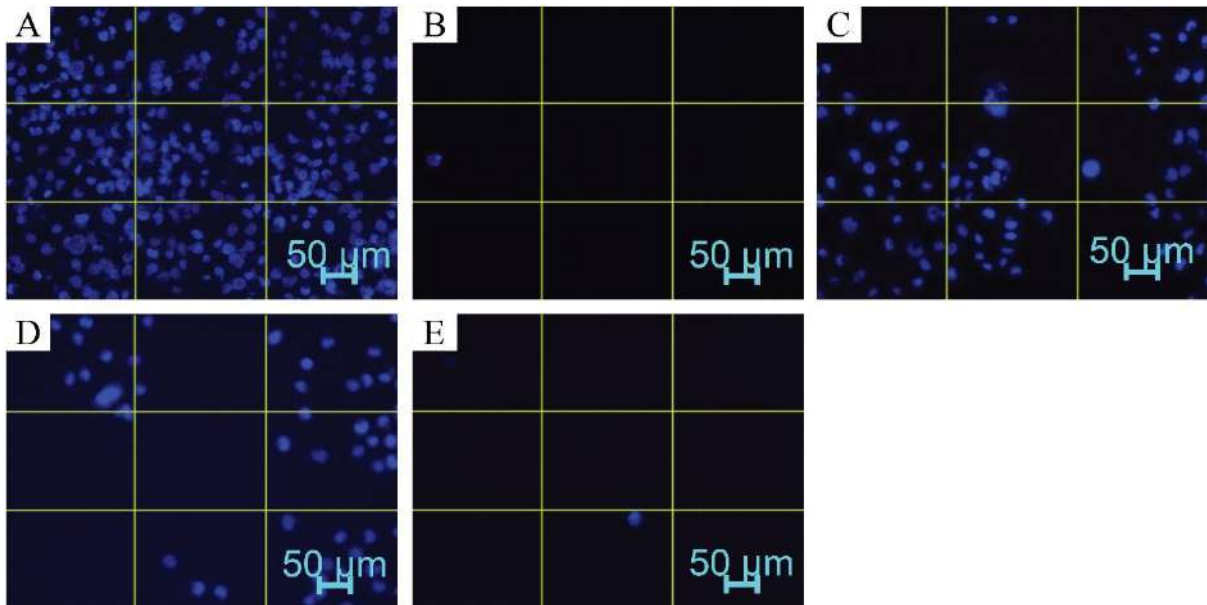
292

293 **Figures**



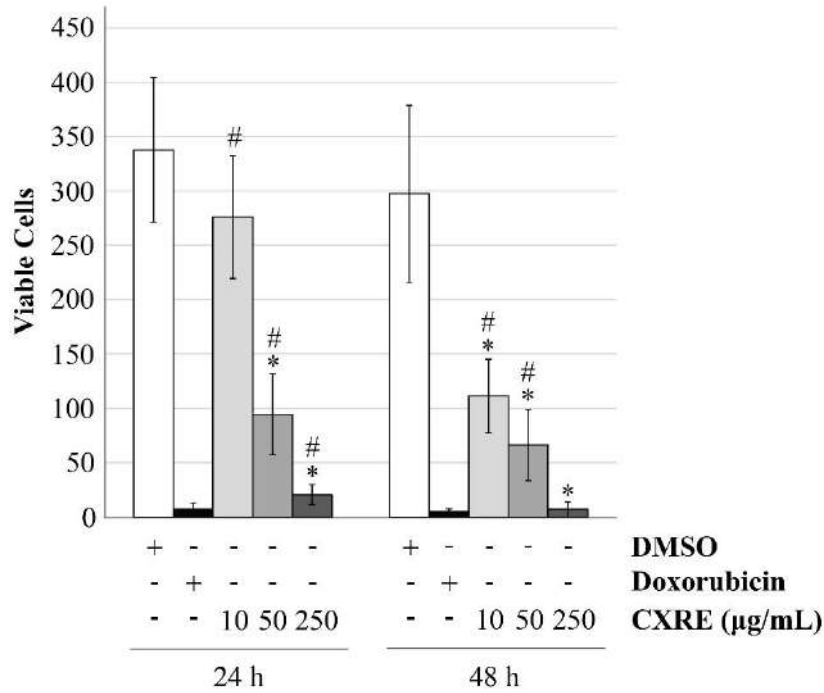
294

295 **Figure 1. CXRE reduced the viability of HONE-1 cells after 24 h.** HONE-1 cells were seeded, starved for 12
 296 h, and treated with DMSO, 3 µM Doxorubicin, or various concentrations of CXRE for 24 h. Cell nuclei were
 297 stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B:
 298 Doxorubicin; C: 10 µg/mL CXRE; D: 50 µg/mL CXRE; E: 250 µg/mL CXRE.



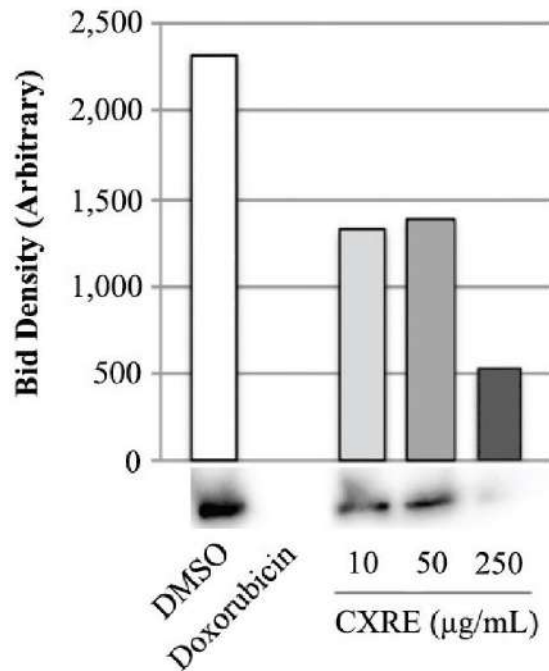
299

300 **Figure 2. CXRE reduced the viability of HONE-1 cells after 48 h.** HONE-1 cells were seeded, starved for 12
 301 h, and treated with DMSO, 3 µM Doxorubicin, or various concentrations of CXRE for 48 h. Cell nuclei were
 302 stained with DAPI as described in Methods. These experiments were repeated three times. A: DMSO; B:
 303 Doxorubicin; C: 10 µg/mL CXRE; D: 50 µg/mL CXRE; E: 250 µg/mL CXRE.



304

305 **Figure 3. CXRE decreased the number of viable HONE-1 cells in concentration- and time-dependent**
 306 **manner.** HONE-1 cells were seeded, starved for 12 h, and treated with DMSO, 3 µM Doxorubicin, or various
 307 concentrations of CXRE for 24 and 48 h. Cell nuclei were stained with DAPI. The number of viable cells in
 308 each slide was evaluated by two independent observers as described in Methods. The data were expressed as
 309 mean ± SD (n=3). **p*<0.05 vs. DMSO-treated group; #*p*<0.05 vs. Doxorubicin-treated group.



310

311 **Figure 4. CXRE diminished Bid expression in HONE-1 cells.** HONE-1 cells were seeded, starved for 12 h,
 312 and treated with DMSO, 3 µM Doxorubicin, or 10, 50, or 250 µg/mL CXRE for 6 h as indicated in the panel.
 313 Cells were collected, lysed, and further processed to obtain cell lysate for Western blot analysis as described
 314 in Methods.



Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2023009 Editor Decision - Manuscript Accepted

Secretariat of InaBJ <secretariat@inabj@gmail.com>
To: ferry@trisakti.ac.id

Fri, Jan 20, 2023 at 7:56 AM

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "***Curcuma xanthorrhiza* Rhizome Extract Induces Apoptosis in HONE-1 Nasopharyngeal Cancer Cells Through Bid.**"

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

Congratulations on your interesting research, and thank you for allowing us to publish this valuable material. Please let us know once you have read this email. We wish you a nice day.

Best Regards,

--

Secretariat of The Indonesian Biomedical Journal

Prodia Tower 9th Floor

[Jl. Kramat Raya No.150, Jakarta 10430, Indonesia](#)

Phone. +62-21-3144182 ext. 3872

Fax. +62-21-3144181

<https://www.inabj.org>