INTRODUCTION

Curcumin, the natural compound of Curcuma spp. had been reported to pose antiproliferative and induce apoptosis on various cancer cell lines [1]. However, it is an unstable compound due to the methylene acetone group [2]. Pentagamavunon-0 (PGV-0) and PGV-1 are analogs of curcumin and were synthesized and patented by Samhoedi et al. (2009). Both compounds were developed to increase the stability compared to curcumin [2, 3].

Previous experiments have shown the aromatic region of curcumin and analogs play an important role for the activity of curcumin and analogs [4, 5]. Curcumin as a parent compound has been investigated for broad spectrum of biological activities. Many experiments showed curcumin could inhibit cancer cell growth by inducing apoptosis and inhibiting the cell cycle progression as reviewed by Raviendran et al. [6].

PGV-0 and PGV-1 have been explored for their biological activities, the experiments showed that PGV-0 and PGV-1 possessed a cytotoxic effect [7, 8]. PGV-1 has a stronger effect to inhibit T47D-cell, which IC50 was 1.74 µg/mL compared to PGV-0 and curcumin, which were 9.39 and 24.97 µg/mL, respectively [9]. PGV-0 and PGV-1 combined with doxorubicin could inhibit MCF-7 cell by apoptosis induction and cell cycle arrest [10].

PGV-0, PGV-1 and curcumin were indicated to inhibit T47D-cell line on G2M phase and induce apoptosis through caspase-3 and caspase-9. The inhibition on G2M phase was independent of the apoptosis process [11]. A further experiment was needed to investigate the effect of curcumin's analogs PGV-0 and PGV-1 on different cancer cell line on normal cell.

METHODS

Materials

HeLa, MCF-7, T47D cancer cells and NIH3T3 normal cell were purchased from E-Merck. All the other reagents were purchased from Sigma-Aldrich and Gibco. The media were DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. Curcumin analogs (PGV-0 and PGV-1) were prepared at Gadjah Mada University, and curcumin was purchased from E-Merck.

Cells growth test

Harvested cells were seeded onto 96 well plates (3,000 cells in each well) in 100.0 µl culture medium, and incubated for 24 hrs in CO2, 37°C. 100.0 µl sample was added with the final concentration of PGV-0, PGV-1 and curcumin were 2.5, 5.0 and 10.0 µM, respectively. The cells were incubated for 6, 12, 24, 48, and 72 hrs. After time incubation, the medium was discarded and cells were washed using PBS pH 7.4 and replaced with 100.0 µl culture medium and added with 15.0 µl MTT (5 mg/ml) and followed by a 6 hrs incubation period. The formazan was diluted in 10% SDS HCl (0.1%). The result was measured using enzyme-linked immunosorbent assay reader (550 nm).
DNA cell staining
Harvested cells (1.5×10^5) was seeded on sterile cover slips in 24 well plates and incubated to get 50% confluence. Cells were incubated with samples for 24 hrs in a 5% CO2 incubator, 37°C. The medium was discarded, and cells were added with ethidium bromide 0.5×0.15% acridine orange in 95% ethanol solution for 5 minutes. DNA morphology was observed under a fluorescent microscope (Zeiss MC-80). The same procedure was conducted for staining using 2-(4-aminodiphenyl)-6-indolcarbamidine dihydrochloride (DAPI) 0.1% in aquabidest.

Western blot analysis
An equal amount of proteins from cell lysates were solubilized five times of the sample buffer and electrophoresed in 10-15% sodium dodecyl sulfate-polyacrilamide gels and transferred to the polyvinylidene diffuoride membrane. The membranes were first incubated with either primer antibody against cleavage PARP, caspase-3, caspase-7 were purchased from cell signaling technology (PUMA (proscience), BAX (abcham), normalized using tubulin (abcham), and then with a horseradish peroxidase-conjugated secondary antibody (cell signaling technology) PUMA and BAX (procience). Western blot analysis was performed on HeLa cell (cervical cancer cell line) and NIH3T3, normal cell line. Curcumin (10.0 µM) just induced apoptosis on T47D-cell and MCF cell but not on HeLa cell (Fig. 4). PARP cleavage resulted by an apoptosis process in the cell [18]. These results also show that PGV-1 (2.5 µM) had a stronger apoptosis effect compared to PGV-0 (5.0 µM) and curcumin (10.0 µM) based on PARP cleavage result qualitatively. On the normal cell (NIH3T3), cells that were treated with the compounds resulted in a negative PARP cleavage. This result indicated that the compounds were selectively induced cancer cell line apoptosis process.

Further investigation of PGV-1 showed apoptosis induction on T47D-cells through caspase-3 activation independent PUMA and BAX. On the other hand, apoptosis induction on MCF-7 cells-even without caspase-3 expression-was related with PUMA and BAX protein. On HeLa cells, apoptosis induction was not related with PUMA and BAX expression and did not depend on caspase-3 activation. These findings indicate that PGV-1 could induce apoptosis on cancer cell lines with normal p53 or mutation of p53. Cells that express p53 normally, apoptosis resulted through an intrinsic pathway and recruited pro apoptosis protein such as PUMA and BAX (Fig. 4).

DISCUSSION
Curcumin, PGV-0 and PGV-1 induced apoptosis on MCF-7 cells. Apoptosis induction on MCF-7 cells indicated that curcumin could induce apoptosis through accumulation of cells on the G2M phase followed by the increase of the p53, p21 expression and release of cytochrome C from the mitochondria [19]. This mechanism is similar with anticancer drugs, Taxol could induce apoptosis through an increase in the level of p53 and p21, which is followed by decreased Bcl-2 and induced activation of caspase-9 [20]. This result supported the previous research that paclitaxel could induce apoptosis through phosphorylated Bcl-2 and increased expression of BAX through activated PKA (cyclic adenosine monophosphate-dependent protein kinase) pathway [21]. N-phenyl-N'-(2-chloroethyl) ureas had been...
Fig. 3: Pentagamavunon-1 (2.5 µg/mL), (a) and PGV-0 (5.0 µg/mL), (b) induced apoptosis on HeLa, T47D and MCF-7. On higher concentration, both compounds could not induce apoptosis on NIH3T3 (normal cell) curcumin, (c) induced apoptosis on T47D and MCF-7 but not on HeLa and NIH3T3, (d) Further investigation of PGV-1 could activate caspase-3 on T47D-cells but not on HeLa and NIH3T3, increased PUMA and BAX on MCF-7 cell but not on HeLa and NIH3T3 cell.

PGV-1 and PGV-0 induced apoptosis on HeLa cells, but not curcumin. Further investigation showed apoptosis on HeLa cells not related with level of PUMA, BAX and caspase-3 activation (Fig. 4). It might be caused by protein E6 that was expressed by HeLa cells that degraded p53 as a transcription factor of PUMA and BAX [13,14]. Apoptosis induction on HeLa cells might involve and extrinsic pathway. Taken together, curcumin analogs could induce apoptosis on T47D, MCF-7 and HeLa cells dependent or independent p53.

Further investigation showed curcumin, PGV-1 and PGV-0 did not induce apoptosis on normal cell NIH3T3. PARP cleavage was not shown after 24 h treatment by those compounds. A higher concentration of PGV-1 also did not induce apoptosis. After 24 h of being induced by PGV-1, NIH3T3 could not activate caspase-3, did not express PUMA, and there was no different level of BAX. All of these results indicated curcumin, PGV-1 and PGV-0 induced apoptosis on cancer cell selectively. Curcumin analogs PGV-0 and PGV-1 are promising to be further developed to support cancer therapy.

CONCLUSION
Curcumin, PGV-0 and PGV-1 could inhibit cells growth by induced apoptosis on cancer cells but not on normal cells, which PGV-1 has the strongest apoptosis induction effect on cancer cell lines.

REFERENCES


