In vitro ultramorphological assessment of apoptosis on CEMss induce by linoleic acid-rich fraction form Typhonium flagelliforme tuber.

ABSTRACT

The plant Typhonium flagelliforme, commonly known as "rodent tuber" in Malaysia, is often used as a health supplement and traditional remedy for alternative cancer therapies, including leukemia. This study aimed to evaluate in vitro anti-leukemic activity of dichloromethane extract/fraction number 7 (DCM/F7) from T. flagelliforme tuber on human T4 lymphoblastoid (CEMss) cell line. The DCM extract of tuber has been fractionated by column chromatography. The obtained fractions were evaluated for its cytotoxicity toward CEMss cells as well as human primary blood lymphocytes (PBLs). Assessment of apoptosis produced by the most active fraction was evaluated by various microscopic techniques and further confirmation of apoptosis was done by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Phytochemical screening was done by gas chromatography-mass spectrometry (GC-MS). The results shows that 7 out of 12 fractions showed significant cytotoxicity against the selected cell line CEMss, in which fractions DCM/F7, DCM/F11 and DCM/F12 showed exceptional activity with 3, 5 and 6.2 μg ml(-1), respectively. Further studies in the non-cancerous PBL exhibited significant selectivity of DCM/F7 compared to other fractions. Cytological observations showed chromatin condensation, cell shrinkage, abnormalities of cristae, membrane blebbing, cytoplasmic extrusions and formation of apoptotic bodies as confirmed collectively by double-staining of acridine orange (AO)/propidium iodide (PI), SEM and TEM. In addition, DCM/F7 has increased the cellular DNA breaks on treated cells. GC-MS revealed that DCM/F7 contains linoleic acid, hexadecanoic acid and 9-hexadecanoic acid. The present results indicate that T. flagelliforme possess a valuable anti-leukemic effect and was able to produce distinctive morphological features of cell death that corresponds to apoptosis.

Keyword: Apoptosis; Leukemia; Linoleic acid; Tryphonium flagelligorm.