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Full Length Research Paper

Effects of cola nut (*Cola nitida*) on the apoptotic cell of human breast carcinoma cell lines

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The cola nut (*Cola nitida*) tree is native to West Africa. It has been naturalized to South America, Central America, the West Indies, Sri Lanka, and Malaysia. Related to cocoa, cola nut is the source of a stimulant, and contains the polyphenolic compounds (including catechin, epicatechin, tannins, and 'Kola red') and the methylxanthine alkaloids that also occur in coffee, cocoa, and tea. Previous study has been shown the potential anticarcinogenic effect of cola nut extract on human breast cancer cell lines, MCF-7.This study was conducted to determine their mechanism of action through the apoptotic cell approach. The effect of cola nut extract on the apoptotic cell of MCF7 cells was determined by flow cytometric analysis. MCF7 cells were treated with 60 and 80 µg/ml of cola nut extract for 24 h and subjectedto FACS analysis. MCF7 cells treated with 60 µg/ml cola nut extract showed an increase of 6.55% in population of apoptotic cells with a concomitant decrease in the percentage of cells in the S and G2/M phase of cell cycle compared to DMSO-treated control cells. Similarly, MCF7 cells treated with 80 µg/ml cola nut extract showed an increase of 8.29% in population of apoptotic cells with a concomitant decrease in the percentage of cells in the S and G2/M phase of cell cycle compared to DMSO-treated control cells. This suggests that cola nut treatment induces apoptosis at 24 h. In conclusion, cola nut extract may induce apoptosis in MCF-7 cell lines.

Key words: *Cola nitida*, apoptosis.

INTRODUCTION

It is now well-accepted that apoptosis (programmed cell death), is a physiological phenomenon that plays an important role in the regulation of tissue development and homeostasis. Deregulation of apoptosis has been shown to contribute to the pathogenesis of a number of human diseases including cancer (Gerschenson and Rotello, 1992; McDonald and El-Deiry, 2000; Hengatner, 2000).

The cola nut tree is native to West Africa. It has been naturalized to South America, Central America, the West Indies, Sri Lanka, Malaysia and Indonesia. Related to cocoa, cola nut is the source of a stimulant, and contains the methylxanthine alkaloids that occur also in coffee, cocoa, and tea. The three varieties of the kola nuts (*Cola nitidaalba*, *Cola nitidarubra* A. Chev, and *Cola acuminata* Schott and Endl) contained appreciable levels of (+)-catechin (27 to 37 g/kg), caffeine (18 to 24 g/kg), (–)-epicatechin (20 to 21 g/kg), procyanidin B_1 [epicatechin-(4β→8)-catechin] (15 to 19 g/kg), and procyanidin B_2 [epicatechin-(4β→8)-epicatechin] (7 to 10 g/kg) (Atawodi et al., 2007). Of the 40 known species, *Cola acuminata* and *Cola nitida* bear the nuts most readily available in the United States and Europe; other species frequently used

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in commerce include *Cola verticillata* and *Cola anomala*. West Africans have been chewing cola nuts for thousands of years. Its stimulant effects are its predominant application in the United States and Europe. In Africa, however, cola nuts have been used as an appetite and thirst suppressant, enabling soldiers who chewed them to travel long distances without much food. Cola twigs, with an extremely bitter taste, are used to clean the teeth and gums (Mitch 2008). Today, cola nut is exported worldwide. It is used in the manufacture of methylxanthine-based pharmaceuticals. Cola nut is also used in non-pharmaceutical preparations, including (at least formerly) cola-based beverages such as Coca cola. It is on the generally recognized as safe (GRAS) list for food additives in the United States (Mitchell, 2008).

Cancer appears to be a major cause of morbidity and mortality and runs in the top three cause of death worldwide especially in the developed countries (WHO, 2009). Chemotherapy is one of the potent treatments for prolonging the patient's life. Almost 60% of anticancer drugs are of natural origin, such as plants that is (vincristine, irinotecan, camptothecines) and microorganisms that is (doxorubicin, dactinomicines, mitomycin and bleomycin) (Grever, 2001). However, many chemotherapeutic drugs are presently faced in a predicament of reduced therapeutic effect due to the problem of drug-resistance (Peters et al., 2002). Moreover, chemotherapeutic drugs also exert toxicity to normal cells which in turn causes the unpleasant side effects to the patients. For these reasons, research and development for new classes of anticancer agents which exhibit efficient and selective toxicity on tumor cells is attracting increased attention.Previous study has been shown the potential anticarcinogenic effect of cola nut extract on human breast cancer cell lines, MCF-7. This study was conducted to determine their mechanism of action through the apoptotic cell approach.

MATERIALS AND METHODS

Plant materials and extraction

The extraction process was done at Laboratory of Biochemistry, School of Medicine, YARSI University, Jakarta, Indonesia. The cola nuts were collected from the herb garden of Karyasari village, Bogor, Indonesia. The cola nuts were extracted by using methanol 80%. A hundred grams of cola were mixed three times with 100 ml of methanol 80%. Extraction continued until the extraction solvents became colourless (total solvents volume were 500 ml). The obtained extracts were filtered over Whatman No.1 paper and the filtrates were collected, then methanol was removed by a rotary evaporator at 50°C.

Culturing of cells

MCF-7 cell lines were obtained from American Type Culture Collection (ATCC, USA). The cells were grown by using Dulbecco's Modified Eagle medium (Gibco, USA). The cells were cultured in their own medium supplemented with 10% of fetal calf serum, 100 IU/ml penicillin and 100 µg/ml of streptomycin (Gibco, USA) using 25 cm^2 flasks (Nunc, Denmark), in a CO_2 incubator (Sanyo, Japan) at 37°C.

Cell cycle analysis by flow cytometry

MCF-7 cells were treated with cola nut extract or DMSO for 24 h. Cells were harvested by trypsinization and permeabilized with ice-cold 70% ethanol for at least 1 h. After washing with phosphate-buffered saline (PBS), the cells were treated with 100 mg/ml of RNase A (DNase free) at 37°C. After 30 min, cells were washed with PBS and stained with 50 mg/ml of propidium iodide for 30 min. DNA contents were analyzed by FACScan (Becton Dickinson, San Jose, CA).

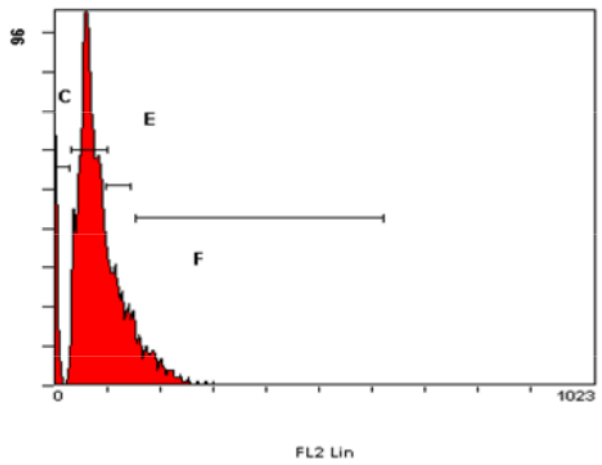
RESULTS

We studied the effect of cola nut extract on cell-cycle distribution of cancer cells by flow analysis. MCF7 cells treated with 60 µg/ml cola nut extract showed an increase of 6.55% in population of apoptotic cells with a concomitant decrease in the percentage of cells in the S and G2/M phase of cell cycle (Figure 1) compared to DMSO-treated control cells (Figure 3).

Similarly, MCF7 cells treated with 80 µg/ml cola nut extract showed an increase of 8.29% in population of apoptotic cells with a concomitant decrease in the percentage of cells in the S and G2/M phase of cell cycle (Figure 2) compared to DMSO-treated control cells (Figure 3). This suggests that cola nut treatment induces apoptosis at 24 h.

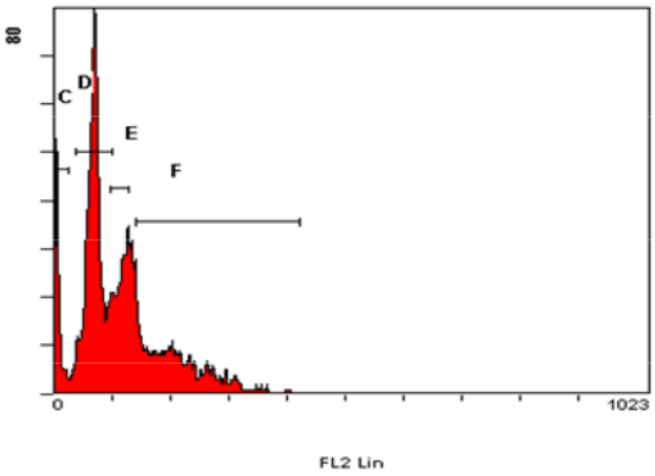
DISCUSSION

Plant-derived compounds have played an important role in the development of several clinically useful anti-cancer agents (Cragg and Newman, 2006). Some of plant-derived anticancer drugs in clinic include the alkaloids vinblastine and vincristine isolated from *Catharanthu sroseus*, topotecan, acamptothecin analogue from *Camptotheca acuminata*, and etoposide andteniposide, semisynthetic analogs of epipodophyllotoxin isolated from *Podophyllum peltatum*. A significant recent addition to this list is taxol, isolated from the bark of *Taxusbrevifolia*. Also, currently several plant-derived pharmacophores are under different phases of clinical development (Rajagopal et al., 2003). The notable examples include flavopiridol a totally synthetic compound based on a flavone isolatedfrom *Dysoxylum binectiferum* (Shapiro et al., 2001), genesteinfrom soyabeans (Wang, 2000), indole 3-carbinol from cruciferous vegetables such as Brussels sprout and broccoli (Bradlow et al., 1999), curcumin from rootof curcuma (Chauhan, 2002), resveratrol from red wine (Bhatand, 2002) and epigallocatechin from green tea (Fujiki et al., 2002), homoharringtonine from the Chinese tree, *Cephalotaxus harringtonia* (Kantarjian et al., 2001), etc. Many anticancer molecules show growth



	%
C	6.55
D	51.15
E	15.61
F	17.03

Figure 1. MCF7 cells treated with 60µg/ml were stained with propidium iodide as described in material and methods and analyzed by flow cytometry. C: apoptotic cells, D: G1 phase, E: S phase, F: G2+M phase.
F1(A and B) 00001370 1037. LMD: PMT3 Lin

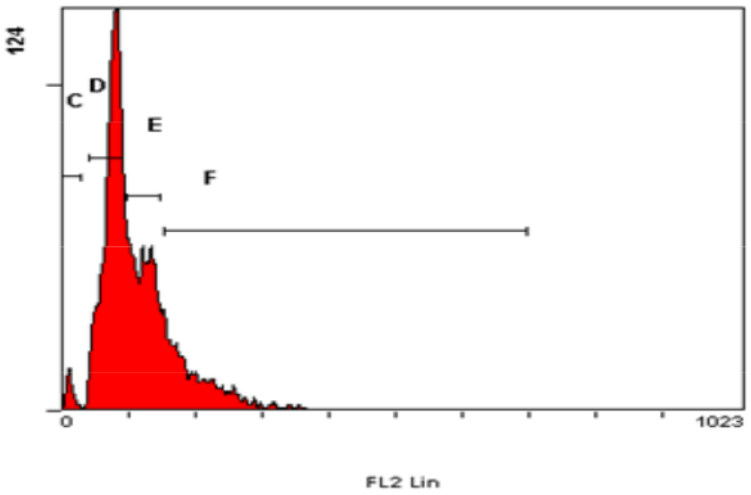


	%
C	8.29
D	38.98
E	15.94
F	30.94

Figure 2. MCF7 cells treated with 80µg/ml were stained with propidium iodide as described in material and methods and analyzed by flow cytometry. C: apoptotic cells, D: G1 phase, E: S phase, F: G2+M phase.
F1 (A and B0 00001339 1013. LMD: PMT3 Lin

inhibition and/or apoptotic cell death of cancer cells by modulating the cell-cycleregulatory molecules directly or indirectly by perturbing the different cell-signaling cascades (Hahn and Weinberg, 2002).

Apoptosis is a form of cell death that permits the removal of damaged, senescent or unwanted cells in multicellular organisms, without damage to the cellular micro-environment. Defective apoptosis represents a major causative factor in the development and progression of cancer. The majority of chemotherapeutic agents, as well as radiation, utilize the apoptotic pathway to induce cancer cell death. Resistance to standard chemotherapeutic strategies also seems to be due to alterations in the apoptotic pathway of cancer cells. Recent knowledge on apoptosis has provided the basis for novel targeted therapies that exploit apoptosis to treat



	%
C	2.46
D	42.53
E	28.29
F	22.58

Figure 3. MCF7 cells treated with DMSO were stained with propidium iodide as described in material and methods and analyzed by flow cytometry. C: apoptotic cells, D: G1 phase, E: S phase, F: G2+M phase.
F1 (A and B) 00001369 1036. LMD: PMT3 Lin

cancer. These new target include those acting in the extrinsic/intrinsic pathway, proteins that control the apoptosis machinery such as the p53 and proteosome pathway (Russo et al., 2006). We studied the effect of cola nut extract on cell-cycle distribution of cancer cells by flow analysis. Cola-treated MCF7 cells showed an increase in population of apoptotic cells with a concomitant decrease in the percentage of cells in the S and G2/M phase of cell cycle. These results suggest that cola nut has the potential for being developed as a cancer therapeutic agent.

Conclusion

The cola nut extract may induce apoptosis in breast cancer cell lines, MCF-7.

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