Short Communication

Beta-sitosterol Induces Apoptosis in MCF-7 Cells

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Abstract

Cyrtandra cupulata Ridl. (Gesneriaceae) is local medicinal plant, also known as *"Bebangun"* or *"Kabut"* by the East Malaysian Ibans. It is indicated for post partum malaise and known to be an abortifacient, among other uses. β-Sitosterol is a known phytoestrogen with many reported bioactivities including anticancer. This study examined the effect of β-sitosterol isolated from *C. cupulata* through bioactivity-guided purification monitored by the growth inhibitory effect on MCF-7, an estrogen receptor-positive breast cancer cell line. Our results demonstrated that β-sitosterol dose-dependently inhibited the growth of MCF-7 cells. Treated cells showed a 1.53-fold increase of DEVDase activity, indicating elevated caspase activity. Thus, the growth inhibitory effect of β-sitosterol isolated from *C. cupulata* appeared to be mediated by caspase-induced apoptosis.

Keywords: beta-sitosterol, apoptosis, MCF-7, *Cyrtandra cupulata*

Introduction

Cyrtandra cupulata Ridl. (Gesneriaceae) is a fleshy herbaceous plant found in the shady under-storey rain forests of Malaysia. A number of plants classified within the genera *Cyrtandra,* including *C. pendula* and *C. oblongifolia,* are used for contraception and to treat post-natal malaise and fever [1], as a postnatal tonic [2] and to induce abortion [3] among the indigenous peoples of Malaysia *(orang asli)* who call it *"Bebangun"* or *"Kabut".* Plants from the same family (Gesneriaceae) have been reported to have beneficial effects on ailments such as menorrhagia and hypermenorrhagia, and even cardiovascular disease [4, 5].

Although the medicinal value of *Cyrtandra cupulata* is known to the indigenous peoples, there is no known documentation of its biological characterization or its mechanisms of action. However, the reported effects are typical of medicinal plants that are known to contain high concentrations of naturally-occurring estrogen-like substances (phytoestrogens). Phytoestrogens have been reported to have many effects in humans, including, the inhibition of cancers [6] and the improvement of brain function [7]. It has been suggested that consumption of phytoestrogen-rich foods might reduce the risk of breast cancer [8]. Dietary phytoestrogens have also been reported to inhibit the *in vitro* proliferation of MCF-7, a breast cancer cell line [9].

β-Sitosterol is a known phytosterol with estrogen-mimetic activity [10]. It is ubiquitous in the plant kingdom but in varying amounts. Commercial β-sitosterol is extracted from corn, soy and plant oils. It has been reported to have antihypercholesterolemic, anti-inflammatory, antibacterial, antifungal and antitumor properties [11].

We tested a purified extract of *C. cupulata,* containing β–sitosterol, on MCF-7 cells in culture. We further investigated the mechanism of the observed inhibition of cellular proliferation.

Materials and Methods

Plant material and isolation of compound

Dried leaves of *Cyrtandra cupulata* were extracted with methanol (yield: 9.32%). Bioactivity-guided purification, monitored by the antiproliferative activity on MCF-7 cells, led to the isolation of β-sitosterol. Briefly, the crude methanolic extract was reconstituted with ultra pure water and subjected to successive liquid-liquid partitioning with hexane, ethyl acetate and butanol (250 ml each). Ethyl acetate fraction (yield: 16.65%) was subjected to absorption column chromatography using silica gel 60 (1 cm x 45 cm) and was eluted with solvent mixtures of increasing polarity (*i.e.,* Hexane : Ethyl acetate, 100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 0:100), followed by methanol (30 ml; washing). A total of 131 fractions (3 ml each) were collected. The fractions were subjected to thin layer chromatography and fractions with similar R_f value compounds were pooled (A_{1-28}). Active fractions were subjected to identification by gas chromatography-mass spectrometry (GC-MS). Beta-sitosterol was identified in fraction A_{11}.

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Activity studied

The antiproliferative activity of β-sitosterol, isolated from *C. cupulata,* on MCF-7 cells was assessed by the MTT assay [12]. Apoptosis was evaluated via cellular caspase activity (*i.e.* DEVDase activity), using a kit (Calbiochem). The substrate, DEVD tetrapeptide, is reactive with caspases 3, 6, 7 and 8 [13]. The assay measures the rate of cleavage of the substrate, acetyl-DEVD-*p*-nitroaniline, to release the chromophore, *p*-nitroaniline (*p*NA).

Results and Discussion

Table 1 demonstrates that β-sitosterol purified from *C. cupulata* inhibited the proliferation of MCF-7 cells. At a concentration of 10 μM, there was a 39% inhibition of proliferation. The cellular DEVDase activity increased significantly ($P < 0.01$) when treated with the fraction: 247.07 pmol *p*NA/min/mg protein (53.00 %), compared to the blank (carrier) at 161.48 pmol *p*NA/min/mg protein. As an internal positive control, the cells were treated with 1 μM of colchicine and exhibited a specific caspase activity of 468.01 pmol *p*NA/min/mg protein (189.83 %). Purified caspase-3 (30 U) provided with the kit was tested along with our sample and was found to have 25.69 ± 2.10 pmol/min, indicating that the assay was working at an acceptable level.

The induction of DEVDase activity in the MCF-7 cells that were exposed to β-sitosterol isolated from *C. cupulata* indicates an elevated general caspase activity. DEVD tetrapeptide is reactive to several caspases [13]. However, the involvement of caspase-3 has been ruled out as MCF-7 cells do not express the enzyme due to the 47-base pair deletion within exon-3 of the caspase-3 gene, resulting in a premature termination of caspase-3 mRNA synthesis [14]. MCF-7 cells that were supplemented with β-sitosterol have been reported to cause an increase in cellular caspase-8 activity [15].

Conclusion

The antiproliferative activity of β-sitosterol on MCF-7 cells is mediated by caspase-induced apoptosis.

Table 1: Antiproliferative and DEVDase activities of β-sitosterol on MCF-7 cells

β-sitosterol		Activities (mean ± SEM; N = 4)	
μM	$\log_{10}$ μM	Antiproliferative [d] (% Inhibition)	DEVDase (pmol/min/mg protein) [b]
0.001	-3	4.74 ± 2.06	- [c]
0.01	-2	16.64 ± 3.29 [a]	- [c]
0.1	-1	31.28 ± 1.19 [a]	- [c]
1	0	37.86 ± 0.98 [a]	- [c]
10	1	39.08 ± 0.79 [a]	247.07 ± 1.69 (1.53 fold [a])

MCF-7 cells were supplemented with β-sitosterol for 3 days.
[a] indicates $p < 0.01$
[b] Baseline DEVDase activity was at 161.48 pmol/min/mg protein, while Colchicine treated MCF-7 cells (+ve control) was at 468.01 pmol/min/mg protein (2.90-fold [a])
[c] Not investigated
[d] Estimated by MTT assay

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