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Induction of apoptosis in human B-lymphoma cells by citral

Chatikorn Bunkrai[1*], Tada Sueblinvong[2], Wacharee Limpanasithikul[3]

[1] Inter-department Program of Pharmacology, Graduate School, Chulalongkorn University.
[2] Department of Biochemistry and [3]Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
[*] Presenting Author

Abstract

This study intended to evaluate anti-tumor activity of citral on human B-lymphoma cell line Ramos. Citral caused Ramos cell death in a concentration-dependent (37.5, 75 and 150 μM) as well as time dependent (3, 6 and 12 h) manner. By staining with annexin V-FITC and propidium iodide (PI) and detecting with flow cytometer, citral induced cell death mostly in apoptotic pattern. Other types of cell death were also detected only when the cells were treated with high concentrations of citral for 6 and 12 h while apoptosis was still dominate. Normal human peripheral blood mononuclear cells (PBMCs) were much more resistant than Ramos cells to apoptotic induction by citral. These results demonstrated the beneficial effect of citral which rich in Thai herbs and spices in oncology.

Keywords**:** citral, apoptosis, Ramos cells

Introduction

Citral, 3,7-dimethyl-2,6-octadienal, is a monoterpenoid containing a mixture of cis-isomer neral and trans-isomer geranial. It is in the volatile oils of many Thai herb and spice such as lemon grass (*Cymbopogon citratus*), lemon balm (*Melissa officinalis*), and *Litsea cubeba*. It is widely used in industries as favoring agent in food, cosmetics and detergent (Opdyke, 1979). Its pharmacological activities have also been studied. It has been reported to have antibacterial (Hayes, 2002), antifungal (Silva, 2008), anti-parasitic (Hierro, 2006) as well as anticancer properties. It inhibited breast cancer cells, MCF-7, growth with IC_{50} 180 μM (48 h) and induced apoptosis in these cell less than 50% at 200 μM (48 h). It also induced apoptosis in many leukemic cell lines at IC_{50} 47 μg/ml (16 h). In our study, the effect of citral on B-lymphoma cell line Ramos was investigated.

Materials and Methods

Chemicals and reagents

Citral was obtained from Sigma-Aldrich (USA). Reagents for cell culture were from Gibco USA. Apoptosis kit for flow cytometer was purchased from Promega, USA.

Cells

Human B-lymphoma cells (Ramos) were obtained from ATCC (USA). Human peripheral blood mononuclear cells (human PBMCs) were prepared from heparinized blood of healthy male blood donors, age 20-35 years old with informed consent at the National Blood Bank, Thai Red Cross Society by ficoll gradient centrifugation. Both cell types were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 0.5% L-glutamine, 100 μg/ml streptomycin and 100 units/ml penicillin, at 37^0C in 5% CO_2/95% air.

Determination of apoptotic induction by citral

Ramos cells or PBMCs at $1x10^6$ cells/ml were treated with citral at the concentrations of 37.5, 75 and 150 µM in 0.5% ethanol for 3, 6 and 12 h. Apoptotic cells were determined by detecting the exposure of phosphatidylserine (PS) on the outer cell membrane by annexin V-FITC and PI staining assay with fluorescence flow cytometer. Mint oil and 0.5% ethanol were used as the negative controls in the study.

Statistical analysis

Data were presented as mean ± S.E. Statistical comparisons were made by one-way ANOVA followed by Tukey's post hoc test according to the statistic program, SPSS version 17. Any p-value < 0.01 was considered statistically significant.

Results

The apoptotic effect of citral on Ramos cells

Citral statistically induced Ramos cell death mostly by apoptosis in the time- (3, 6 and 12 h) and concentration- (37.5, 75 and 150 µM) manner (Table 1 and Figure 1). Non-apoptotic cell death was detected only at the high concentrations of citral (75 and 150 µM) for longer time exposure (12 h), but apoptosis was still the main type of cell death. Ten µg/ml etoposide slightly induced cell death at 3-12 h of exposure but it caused 45% cytotoxicity to Ramos cells at 24 h exposure (data not shown).

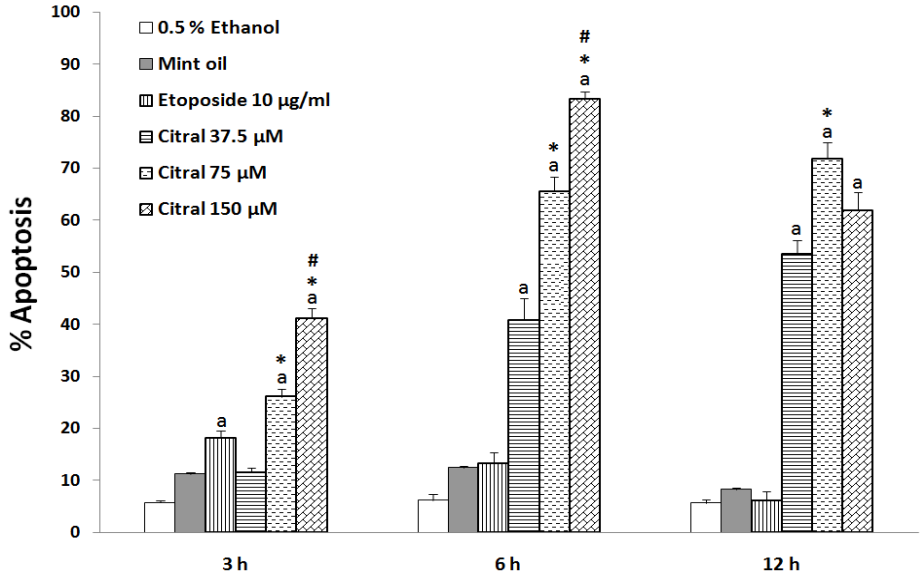

Figure 1. Effect of citral on Ramos cells apoptosis. Ramos cells ($1x10^6$ cells/ml) were treated with 37.5, 75 and 150 µM citral for 3, 6 and 12 h. Mint oil and 0.5% ethanol treated cells were the negative controls while 10 µg/ml etoposide was used as the positive control. The treated cells were stained with annexin V-FITC/PI. The types and the percentage of cell death were detected by fluorescence flow cytometer. Apoptotic cells were detected as annexin V-FITC positive cells. The data are expressed as mean ± S.E. of three independent experiments (n=3). a p < 0.01 significantly different from 0.5% ethanol, * p < 0.01 significantly different from 37.5 µM citral, and # p < 0.01 significantly different from 75 µM citral.

Table 1. Effect of citral on Ramos cell death.

	% Cell death					
	3 h		6 h		12 h	
	Total death	**Apoptosis**	**Total death**	**Apoptosis**	**Total death**	**Apoptosis**
0.5% Ethanol	6.17 ± 0.38	5.73 ± 0.37	6.63 ± 1.15	6.22 ± 1.05	5.83 ± 0.59	5.63 ± 0.60
Mint oil	12.83 ± 0.45	11.20 ± 0.35	13.30 ± 0.28	12.42 ± 0.41	12.87 ± 1.77	8.23 ± 0.39
Etoposide 10 µg/ml	19.20 + 1.07	18.23 ± 1.25	15.22 ± 2.47	13.17 ± 2.18	26.03 ± 1.50	6.10 ± 1.85
Citral 37.5 µM	12.33 ± 0.80	11.50 ± 0.85	43.20 ± 4.69	40.72 ± 4.30	68.37 ± 4.37	53.53 ± 2.62
Citral 75 µM	27.43 ± 1.10	26.07 ± 1.44	70.83 ± 2.87	65.47 ± 2.91	93.07 ± 1.47	71.83 ± 3.16
Citral 150 µM	43.03 ± 1.90	41.10 ± 1.93	90.57 ± 2.28	83.30 ± 1.49	98.87 ± 0.03	61.97 ± 3.37

Note: Ramos cells ($1x10^6$ cells/ml) were treated with 37.5, 75 and 150 µM citral for 3, 6 and 12 h. Mint oil and 0.5% ethanol treated cells were the negative controls while 10 µg/ml etoposide was used as the positive control. The treated cells were stained with annexin V-FITC/PI. The types and the percentage of cell death were detected by fluorescence flow cytometer. Apoptotic cells were detected as annexin V-FITC positive cells.

The apoptotic effect of citral on PBMCs

Citral had no cytotoxic effect on PBMCs after 3 h of treatment. It induced cell death only in apoptosis pattern, in a concentration- and time-dependent manner, at 6 and 12 h of exposure (Figure 2). Its effect on PBMCs was much less than on Ramos cells after 3 and 6 h of treatment. It had less than 50% cytotoxic to the normal cells at 150 µM for 12 h exposure, but this effect was still less than its effect on Ramos cells which is 98.87 ± 0.03% cytotoxicity.

Discussion and Conclusion

Citral is a volatile oil presents in several Thai herbs and spices such as lemon grass (65-85%). We demonstrated that citral induced cell death mainly by apoptosis in human B-lymphoma cells, Ramos cells, in a concentration- and time-dependent manner. Ramos cell apoptosis was significantly detected after 3 h exposure to 75 and 150 µM citral. Citral at both concentrations caused more than 90% Ramos cell death after 12 h exposure. It also induced almost 70% apoptosis after 12 h exposure at 37.5 µM. Etoposide at 10 µg/ml had little cytotoxicity on the cells at 3-12 h exposure. It has been reported that citral exhibited cytostatic in MCF-7 breast cancer cells. It inhibited these cell proliferation with IC_{50} 180 µM after 48 h exposure (Chaouki, 2009). It induced MCF-7 cell death by 50% after 48 h exposure at 200 µM. Citral also induced apoptosis in other hematopoietic cancer cell lines including BS 24-1, Jurkat and U937 with IC_{50} 47 µg/ml at 16 h exposure (Dudai, 2005). We also evaluated the selectivity and safety of citral. Citral at 37.5 µM had 68% cell death on Ramos cells without any cytotoxicity on normal human PBMCs after 12 h exposure. Apoptotic effect of citral on Ramos cells was much higher than on normal blood cells, especially at the short time of exposure, 3 and 6 h.

The results from our study demonstrated that citral induces human B-lymphoma cell apoptosis within a short time of exposure. Citral may be developed as an agent for the management of lymphoma cancer. However, more in depth studies both *in vitro* as well as *in vivo* in appropriate relevant animal models are needed to strengthen this suggestion.

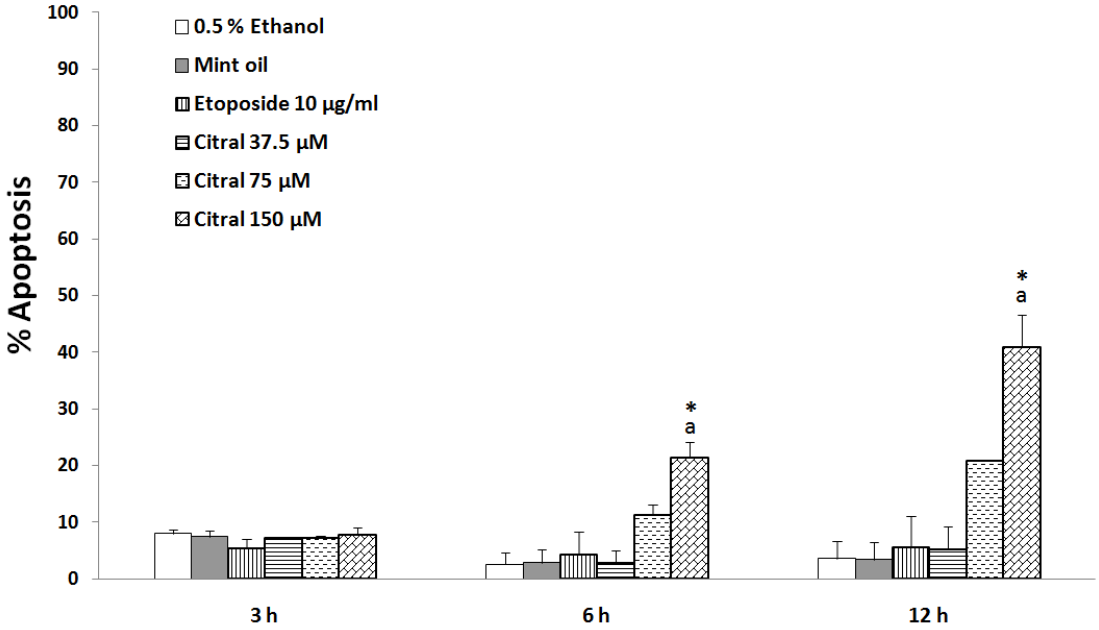

Figure 2. Effect of citral on normal cells (human PBMCs). PBMCs ($1x10^6$ cells/ml) were treated with 37.5, 75 and 150 µM citral for 3, 6 and 12 h. Mint oil and 0.5% ethanol treated cells were the negative controls while 10 µg/ml etoposide was used as the positive control. The treated cells were stained with annexin V-FITC/PI. The types and the percentage of cell death were detected by fluorescence flow cytometer. Apoptotic cells were detected as annexin V-FITC positive cells. The data are expressed as mean ± S.E. of three independent experiments (n=3). a $p < 0.01$ significantly different from 0.5% ethanol, * $p < 0.01$ significantly different from 37.5 µM citral.

Acknowledgments

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