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Anticancer Effects of 23-Dihydroganoderic Acid N

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ABSTRACT : 23-Dihydroganoderic acid N, a triterpenoid compound, was investigated whether it may show cytotoxic activity against U937, HeLa, NCI-H460 and MCF-7 cancer cells by MTT test. As a result, 23-dihydroganoderic acid N show sensitive to MCF-7 cells among NCI-H460, HeLa, U937, and MCF-7 cells.

Key words : **Triterpenoids, Anticancer effect, Cytotoxicity**

Introduction

Ganoderma lucidum is widely used in traditional Chinese medicine. In southeast Asian region, such as China, Korea, and Japan, it is used to treat many diseases, including hepatitis, hypertension, hypercholesterolemia and gastric cancer (Mizuno *et al.*, 1995). Its bioactive substances are mainly triterpenes, which have been isolated from its fruiting body, mycelia and spores (Bao *et al.*, 2002; Min *et al.*, 1998, 2000, 2001).

New triterpenoids, ganoderic acids, were isolated from the dried fruiting bodies of *Ganoderma lucidum*. Their structures were elucidated by spectral and chemical transformation studies. The ganoderic acids have been widely studied and shown many interesting biological activities, including anti-complement, anti-inflammatory, anti-HIV-1 protease, inhibition of histamine release and anti-tumor effects (Hong *et al.*, 2004; Kohda *et al.*, 1985; Komoda *et al.*, 1989; Min *et al.*, 1998, 2000, 2001). 23-Dihydroganoderic acid N is a ganoderic acid compound isolated from *Ganoderma lucidum*.

In present study, 23-dihydroganoderic acid N was investigated whether it may show cytotoxic activity against U937, HeLa, NCI-H460 and MCF-7 cancer cells by MTT test.

Materials and Methods

Compound

23-Dihydroganoderic acid N was obtained from Dr. Sam Sik Kang (Seoul National University, Seoul, Korea).

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Reagents

MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide) was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Cell culture

To study the cytotoxic effect of 23-dihydroganoderic acid N against cancer cells, NCI-H460 (human non-small cell lung), HeLa (human cervix cancer cell), U937 (human monocytic leukemia cell) and MCF-7 (human breast cancer cell) from the American Type Culture Collection were used. These cancer cells were maintained at 37°C in an incubator with a humidified atmosphere of 5% CO_2 and were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

Cell viability

The cell viability of 23-dihydroganoderic acid N against various cancer cells are determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells (Carmichael *et al.*, 1987). 23-Dihydroganoderic acid N were treated and incubated for 24 h. The MTT solution was added and incubated for 4 h. The formazan crystals in each well were dissolved in 150 ul dimethylsulfoxide and the absorbance was measured at 540 nm.

Statistical analysis

All the measurements were made in triplicate and all

Table 1. Cytotoxic effect of 23-dihydroganoderic acid N against NCI-H460 cells

Triphlorethol-A (μg/ml)	NCI-H460
0	100.0 ± 7.0%
10	84.1 ± 2.2%
20	84.0 ± 1.7%
40	72.2 ± 2.7%*
60	74.5 ± 3.3%*

Cytotoxic effect of 23-dihydroganoderic acid N against NCI-H460 cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

Table 2. Cytotoxic effect of 23-dihydroganoderic acid N against HeLa cells

Triphlorethol-A (μg/ml)	HeLa
0	100.0 ± 1.6%
10	88.4 ± 1.3%*
20	84.5 ± 3.4%*
40	79.1 ± 1.9%*
60	69.0 ± 1.7%*

Cytotoxic effect of 23-dihydroganoderic acid N against HeLa cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

values were represented as means ± standard error. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. $p < 0.05$ were considered significantly.

Results and Discussion

To find the anticancer compounds from *Ganoderma lucidum*, 23-dihydroganoderic acid N, was isolated. It was detected using MTT assay whether 23-dihydroganoderic acid N have cytotoxic effect against various cancer cells. As shown in Table 1, 23-dihydroganoderic acid N at 10, 20, 40, 60 μg/ml showed the cell viability of 84%, 84%, 72% and 75% against NCI-H460 cells, respectively. In HeLa cells, it showed the cell viability of 88%, 85%, 79% and 69%, respectively (Table 2). And 23-dihydroganoderic acid N at 10, 20, 40, 60 μg/ml showed the cell viability of 82%, 81%, 74% and 71% against U937 cells (Table 3), respectively. In MCF-7 cells, it showed the cell viability

Table 3. Cytotoxic effect of 23-dihydroganoderic acid N against U937cells

Triphlorethol-A (μg/ml)	U937
0	100.0 ± 3.3%
10	82.0 ± 0.7%*
20	80.6 ± 4.1%*
40	73.7 ± 1.7%*
60	70.5 ± 4.0%*

Cytotoxic effect of 23-dihydroganoderic acid N against U937cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

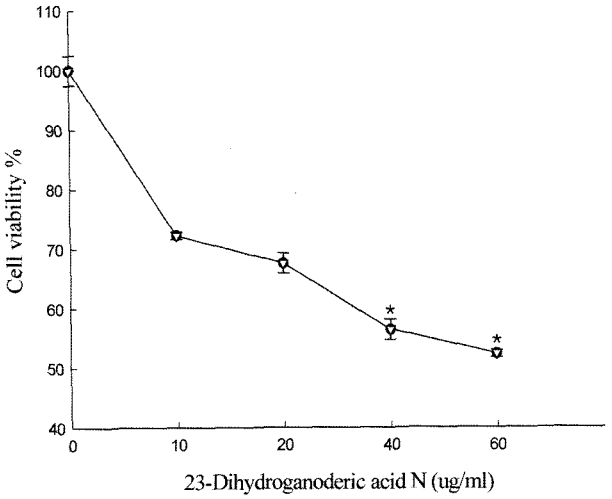

Fig. 1. Cytotoxic effect of 23-dihydroganoderic acid N against MCF-7 cells. The viability was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± S.E. *significantly different from control ($p < 0.05$).

of 72%, 68%, 56% and 52%, respectively (Fig. 1). Therefore, 23-dihydroganoderic acid N shows sensitive to MCF-7 cells among NCI-H460, HeLa, U937, and MCF-7 cells.

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