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Anticancer and Antioxidant Effects of Bioactive Extracts from Monk Fruit (*Siraitia grosvenori*) with Potential Clinical Implications

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ABSTRACT

Background: *To find the better option for cancer treatment and prevention, we have been working on the bioactive extracts of monk fruit, mogrosides, with potential anticancer and antioxidant activities. As oxidative stress (OXS), generation of reactive oxygen species, is believed to play a significant role in carcinogenesis, certain antioxidants may help prevent the development of cancer. Accordingly, we investigated if such mogroside-based products would have such biological activities in vitro.*

Materials and Methods: *Anticancer effect of four selected products, LS, LME, and LLE, and MOG, was tested on five different cancer cells, including bladder, prostate, breast, lung, and liver. Cancer cells were treated with these products for 72 h and cell viability was determined by MTT assay. The anticancer mechanism was also explored, focusing on cell cycle and apoptosis. For antioxidant study, whether any products would protect normal kidney cells from hydrogen peroxide (H_2O_2)-induced OXS was examined. Cell viability and severity of OXS were determined by MTT assay and lipid peroxidation (LPO) assay, respectively.*

Results: *Although any concentrations (0-500 μg/ml) of LS and LME had little effects, LLE (≥2 μg/ml) and MOG (≥1.5 mg/ml) showed a significant cell viability reduction in all five cancer cells. LLE and MOG, not LS and LME, concurrently led to a G_1 cell cycle arrest and ultimately apoptosis. Additionally, LLE and MOG also protected normal cells from H_2O_2-induced OXS, and LPO assay further revealed a significant reduction in the severity of OXS with them.*

Conclusions: *In this study, two of four monk fruit products tested, LLE and MOG, demonstrated their anticancer and antioxidant activities. They may stop/reduce cancer cell growth/viability, while they may also protect normal cells from OXS that can lead to the cancer development. Therefore, these two monk fruit products may have clinical implications in the cancer prevention/treatment.*

Keywords

Anticancer, Antioxidant, Cancer, Mogrosides, Monk fruit, Oxidative stress.

Introduction

Cancer is yet the serious dismal disease affecting millions of people in the United States and worldwide. Nearly 1.9 million new cancer cases and approximately 610,000 cancer deaths are estimated this year [1]. Although cancer deaths have declined continuously from 1991 (at the peak) to 2018 [1], the actual number predicted for this year is yet too high to be accepted. Chemotherapy is currently the widely used regimen for cancer patients but suffers from the disappointing efficacy/outcomes with inevitable side effects and drug resistance [2], demanding the more effective and safer therapeutic option. Hence, it is far from over and we must continue the battle against this dreadful disease.

In conjunction with cancer, *oxidative stress (OXS)*, i.e. generation of reactive oxygen species (ROS) [3], is another serious health threat to us. ROS, including superoxide anion, hydroxyl radicals, hydrogen peroxide etc., are highly toxic and primarily generated as byproducts via the incomplete reduction of oxygen during oxidative phosphorylation in mitochondria (an aerobic metabolic process) [4]. Due to such an extremely reactive nature of ROS molecules, OXS has been shown to exert adverse effects on a variety of cells, leading to certain chronic and degenerative diseases, cancers, and may even facilitate the aging-process as well [5,6]. Hence, as OXS could trigger the cancer development anytime, it should be kept away as much as possible. Unfortunately, it is yet inevitable as long as we keep breathing and generating ATP.

However, there is a good news that *antioxidants* have been reported to have beneficial or protective effects on cellular injury/damage associated with OXS [7]. A number of chemical antioxidants, such as vitamins (Vitamin C and E etc.), coenzyme Q10 (CoQ10), reduced glutathione (GSH) etc. are commercially available as supplements. Instead, we are rather interested in *natural* antioxidants or *natural products* with antioxidant activity. Those include phenolic acid, flavonoids, anthocyanins etc., which mostly come from fruits, flowers, seeds, vegetables, mushrooms etc. [5,8-11]. While they would protect your body from OXS, they also have low toxicity and few side effects. In fact, the medicinal aspects of various *natural products* are rapidly receiving public attention and their global sales have steadily increased in the past 20 years [12,13]. Moreover, those products have been accepted as a non-mainstream practice in Complementary and Alternative Medicine (CAM), which was now endorsed by the medical and scientific communities [14]. Hence, we have been seeking for any natural products with *antioxidant* activity as well as *anticancer* activity (against cancer) because they may offer the alternative, improved, and safer regimens.

We then came across the bioactive extracts of monk fruit (*Siraitia grosvenori*) [15] (Figure 1A) whose name is believed to come from an anecdote that Buddhist monks in China have used this fruit as a sweetener for teas or cooking. It has been also used as a folk medicine for cough, sore throat, bronchitis etc. [16]. Indeed, it gives such high sweetness, i.e. 200-350 times sweeter than sucrose [17], which primarily comes from "mogrosides", terpenoid glycosides. They are active ingredients of monk fruit, having several derivatives (mogroside I-VI) based on variations in the position and the number of the glucose moieties attached to a mogrol group [15,18] (Figure 1B). Mogrosides have been purified/extracted and widely used for commercial dietary products as the US Food and Drug Administration (FDA) had approved them for Generally Recognized As Safe (GRAS) for its intended use as a food sweetener [19].

Apart from their sweetness, a number of scientific/medical studies on mogrosides have revealed their potential medicinal/pharmacological properties. Those include anticancer, anticarcinogenic, antioxidant, anti-diabetic, anti-inflammatory, anti-allergic, lipid-lowering activities etc. [17,20-27]. For instance, anticancer effects of mogrosides were demonstrated in colorectal and throat cancers [21], while their antioxidant activity was reported that palmitic acid-induced oxidative stress on pancreatic β-cells was significantly reduced with mogrosides [24].

Strictly speaking, among several mogroside derivatives, *mogroside V* is the most abundant and the sweetest one that has been widely commercialized and used in food products [18]. One of such products for public consumption is called "Lakanto®", which has been developed by the Japanese company (Saraya Co., Ltd., Osaka, Japan). All Lakanto® products are the proprietary products, consisting of purified mogroside V (MOG) and erythritol (sugar alcohol) mixed with a specific ratio, and they are commercially available in a powder and liquid form. However, these products have not yet been fully studied in terms of medicinal/pharmacological properties.

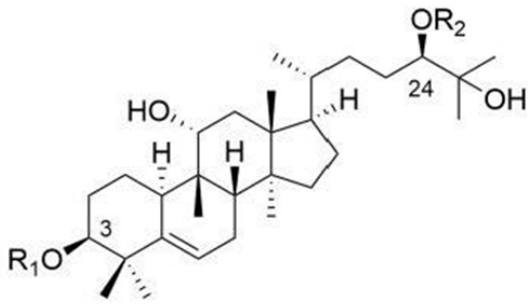
Accordingly, we investigated if four kinds of the Lakanto® products might have anticancer and antioxidant activities with potential clinical implications. Five different types of cancer cells were employed for examining anticancer effect of these products, while three types of normal (non-cancerous) kidney cells were used for assessing their antioxidant effect. More details are described and the interesting findings are discussed herein.

Materials and Methods

Cell Culture

Five different human cancers cells, bladder (T24), prostate (PC-3), breast (MDA-MB231), lung (A549), and liver (HepG2), and additionally three normal kidney cells, LLC-PK_1, MDCK, and OK, were all obtained from the American Type Culture Collection (ATCC; Manassas, VA). They were cultured in RPMI 1640 medium

Figure 1: (A) Monk fruit grown in the trees, ripened, and powdered.



Mogroside IIe: R_1=β-D-glu, R_2=β-D-glu
Mogroside III: R_1=β-D-glu, R_2=β-D-glu-(1→6)β-D-glu
Mogroside IIIe: R_1=β-D-glu, R_2=β-D-glu-(1→2)β-D-glu
Mogroside IV: R_1=β-D-glu-(1→6)β-D-glu, R_2=β-D-glu-(1→2)β-D-glu
Mogroside V: R_1=β-D-glu-(1→6)β-D-glu, R_2=β-D-glu-(1→6)β-D-glu
(1→2)β-D-glu

Figure 1: (B) Basic structure of mogrosides.

supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). Possible anticancer effect of four commercial Lakanto® products, *Lakanto® Sweetener (LS)*, *Lakanto® 50% Monk fruit Extract (LME)*, *Lakanto® Liquid Extract (LLE)*, and *purified MOG*, were examined on five cancer cells above. All these products were generous gifts from a manufacturer (Saraya Co., Ltd. via Saraya International, Inc., Hartsdale, NY). Antioxidant effect of these products were also assessed by chemically induced OXS on three kidney cells. Experimentally, anticancer and antioxidant effects of four products were determined by cell viability (MTT) and lipid peroxidation (LPO) assays described below.

MTT (Cell Viability) Assay

Five different cancer cells, T24, PC-3, MDA-MB231, A549, and HepG2, were seeded in 6-well plates (2 ml/well) or T-75 flasks (10 ml) at the initial cell density of $1x10^5$ cells/ml and were cultured with varying concentrations of four Lakanto® products for 72 h. Cell viability was then determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay following the vendor's protocol (Sigma-Aldrich, St. Louis, MO). As cell viability shows the % of viable cell number in treated-cells relative to that in control cells (100%), anticancer effect can be assessed by "the greater cell viability *reduction*, the *greater* anticancer activity". After cancer cells in the plate were treated with given products for 72 h, MTT reagent (1 mg/ml) was added to each well in the plate that was then incubated for 3 h in an incubator. Dimethyl sulfoxide (DMSO) was added to the plate to dissolve formazan precipitates (purple) and absorbance of samples was read in a microplate reader. Cell viability was then expressed by the % of sample readings (OD) relative to the control reading (100%).

Cell Cycle Analysis

Various cancer cells treated with given agent for 72 h were harvested and subjected to cell cycle analysis. Cells (~1 x 10^6 cells) were first resuspended in propidium iodide solution, followed by a 1-h incubation at room temperature. Approximately 10,000 nuclei from each sample were then analyzed on a FACScan flow cytometer (Becton-Dickinson, Franklin Lakes, NJ), equipped with a double discrimination module. CellFit software was used to quantify cell cycle compartments to estimate the % of cells distributed in the different cell cycle phases, designated as a G_1, S, and G_2/M phase.

Lipid Peroxidation (LPO) Assay

Possible antioxidant activity of four products was assessed against chemically induced OXS on three normal kidney cells, LLC-PK_1, MDCK, and OK. Hydrogen peroxide (H_2O_2)-induced OXS was exerted on these kidney cells for 6 h, and the severity of OXS was determined by measuring the amount of malondialdehyde (MDA) formed as a by-product of oxidative stress [28] – the *greater* MDA formed, the *greater* OXS. Hence, it is also true that the *lesser* MDA formed, the *greater* antioxidant activity, if any of four products have such antioxidant activity. Kidney cells exposed to H_2O_2 alone or with four products for 6 h were subjected to LPO assay described in the vendor's protocol (ABCAM, Cambridge, MA). The amounts of MDA formed with experimental conditions were measured by µM using the MDA standard run and converted to arbitrary values relative to controls (1.0).

Western Blot Analysis

Whether anticancer effect of any Lakanto® products would be linked to apoptosis, (programmed cell death) was examined because such induction of apoptosis may have clinical implications. Briefly, an equal amount of proteins (10 µg) obtained from control and product-treated cell lysates was resolved by 10% SDS-PAGE (SDS-polyacrylamide gel electrophoresis) and transferred to a nitrocellulose membrane (blot). The blot was first incubated for 90 min with the primary antibodies against bcl-2 or Bax (Santa Cruz Biotechnology, Santa Cruz, CA), followed by incubation with the appropriate secondary antibody conjugates (Santa Cruz Biotechnology) for 30 min. After discarding antibodies, the specific immunoreactive proteins (bcl-2 or Bax) were then detected by chemiluminescence (Kirkegaard and Perry Laboratories, Gaithersburg, MD) on an X-ray film (autoradiography).

Statistical Analysis

All data are presented as the mean ± SD (standard deviation), and

statistical differences between groups are assessed with either one-way analysis of variance (ANOVA) or the unpaired Student's t test. Values of $p < 0.05$ are considered to indicate statistical significance.

Results

Effects of Lakanto® Products on Cell Viability of Various Cancer Cells.

Five different cancers cells (1-2 x 10^5 cells/ml) including bladder (T24), prostate (PC-3), breast (MDA-MB231), lung (A549), and liver (HepG2) cancer cells were treated with the varying concentrations of four different Lakanto® products described earlier, LS, LME, LLE, and MOG. At 72 h, cell viability, i.e. the percent (%) of viable cancer cells following treatment of those products, was determined by MTT assay. We define anticancer effect as a *reduction (%)* in cell viability with any of products (compared to control as 100% cell viability).

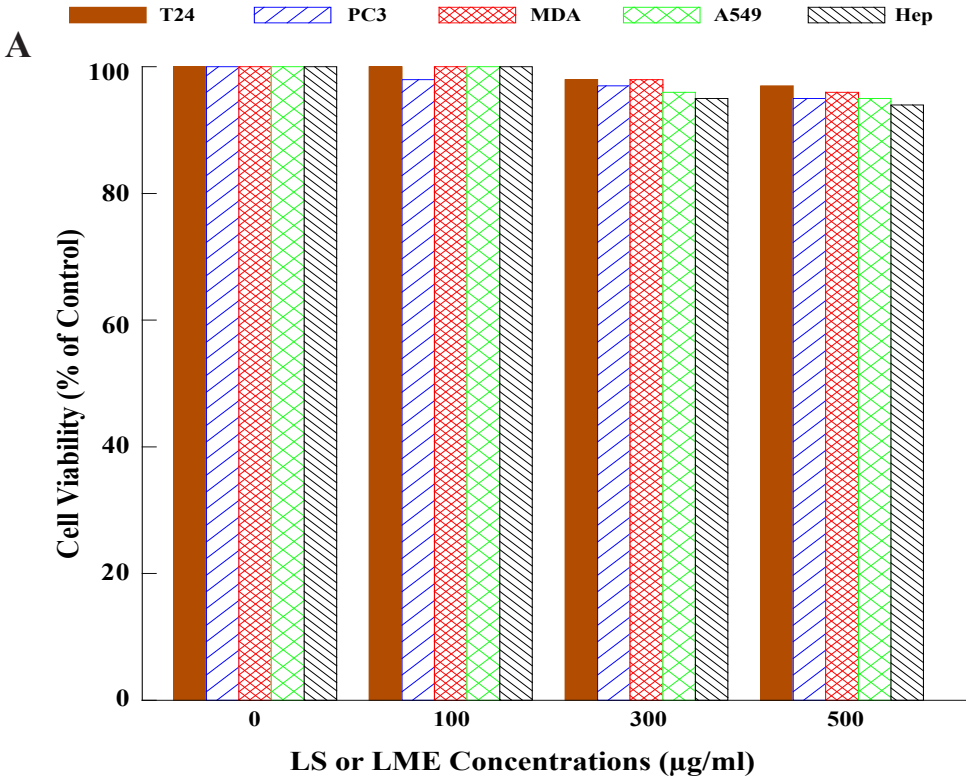


Figure 2: (A) Effects of two Lakanto® products, LS and LME on cell viability of five different cancer cells (T24, PC-3, MDA-MB231, A594, and HepG2). Cells were treated with LS or LME (0-500 µg/ml) for 72 h and cell viability was determined by MTT assay. Cell viability was assessed by the % of viable cell numbers relative to that in control cells (100%).

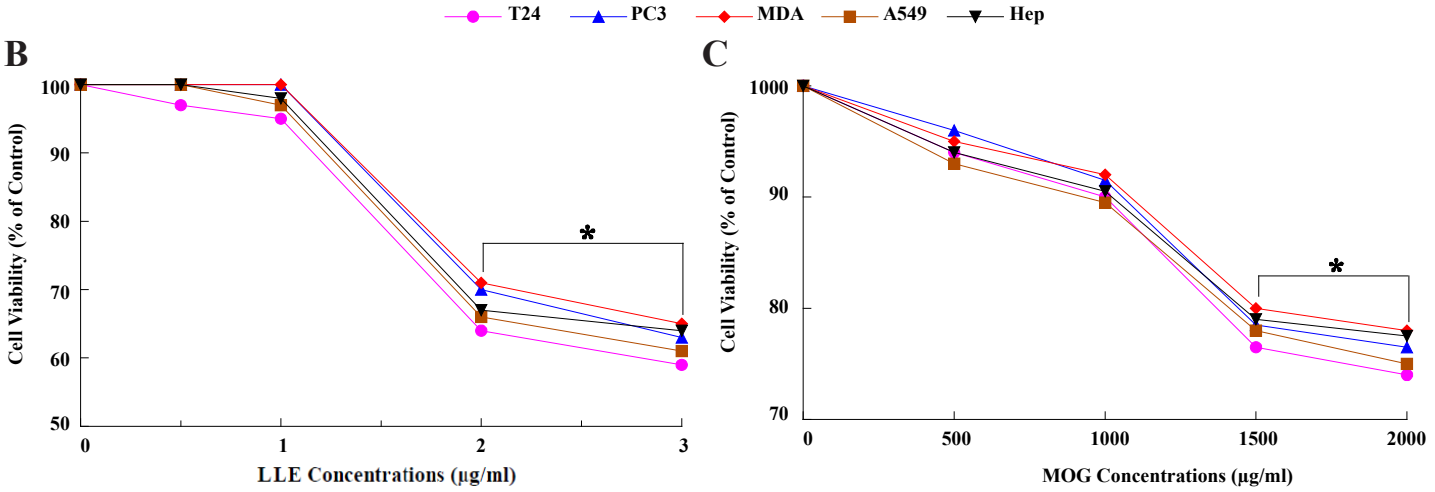


Figure 2: Effects of LLE **(B)** or MOG **(C)** on cancer cell viability. Cells treated with LLE (0-3 µg/ml) or MOG (0-2,000 µg/ml) for 72 h were subjected to MTT assay. All data represent the mean ± SD (standard deviation) from three independent experiments (*$p < 0.05$ compared with controls).

The concentrations of LS and LME up to 500 µg/ml had essentially the same results with little to no effects on cell viability, which are plotted together (Figure 2A). In contrast, the concentrations of LLE ≥2 µg/ml led to a 30-40% reduction in cell viability, demonstrating 30-40% anticancer effects (Figure 2B). The fourth product, MOG, was tested at the relatively high concentrations (0-2,000 µg/ml) because it has been reported to require the high concentrations (>1,000 µg/ml) to be effective [21]. Although little reduction in cell viability was seen up to 1,000 µg/ml, a significant reduction became apparent at 1,500 µg/ml and then cell viability was reduced by 20-26% ($p < 0.05$) with 2,000 µg/ml (Figure 2C). Thus, LLE and MOG have anticancer effect on all five cancer cells, while LS and LME have little effects and were omitted from the rest of our study.

Effects of LLE and MOG on Cell Cycle

To have a better understanding of the anticancer mechanism(s) of LLE and MOG, we looked into their possible effects on cell cycle that would regulate cell division and cell growth [29]. Five cancer cells were treated with LLE (3 µg/ml) or MOG (2,000 µg/ml) for 72 h and subjected to cell cycle analysis. Compared to respective control cells, the G_1-phase cell population *increased* while the S-phase population *decreased* significantly in all cancer cells treated with LLE or MOG (Figure 3). For instance, the G_1 population *increased* from 49.3% in T24 (control) cells to 70.2% in those treated with LLE, whereas the S population *decreased* from 38.6% (control) to 21.2% (LLE-treated) (Figure 3). This accumulation of cells in the G_1 phase is known as a G_1 cell cycle arrest [30], which would eventually lead to the growth cessation and cell viability reduction. Thus, such a cell cycle arrest may at least account for the anticancer mechanism of LLE and MOG.

Induction of Apoptosis in Cancer Cells with LLE and MOG

It was also interesting to address if the cell viability reduction with LLE or MOG would result in apoptosis (programmed cell death). Cells treated with LLE (3 µg/ml) or MOG (2,000 µg/ml) for 72 h were subjected to Western blot analysis for the two specific apoptotic regulators, bcl-2 and Bax. Compared to respective control cells, the bcl-2 expression was *down*-regulated (decreased) while the Bax was *up*-regulated (increased) with LLE or MOG treatment in all five cancer cells (Figure 4). Since bcl-2 is an anti-apoptotic regulator and Bax is a pro-apoptotic regulator [31], the protein pattern with *decreased bcl-2* and *increased Bax* (with LLE or MOG) rather indicates induction of apoptosis. Thus, LLE or MOG appear to ultimately induce apoptosis in cancer cells.

Adverse/Cytotoxic Effects of Oxidative Stress (OXS) on Normal Cells

We next examined if any of four Lakanto® products might have *antioxidant* activity. In this study, OXS was chemically induced by hydrogen peroxide (H_2O_2), one of typical ROS [4], and exerted on three normal kidney cells, LLC-PK_1, MDCK, and OK cells. After cells were treated with the varying concentrations of H_2O_2 (0-80 µM) for 72 h and cell viability was determined by MTT assay. As shown in Figure 5, H_2O_2 was indeed cytotoxic to these normal cells, resulting in a significant reduction in cell viability. Actually, the responses of cells (to H_2O_2) somewhat varied as the IC_{50} values, the concentrations required for inducing a 50% cell grwoth inhibition, were found to be somewhere between 45 and 65 µM. More specificcally, the IC_{50} of 65, 60, and 45 µM (H_2O_2) were then used for LLC-PK_1, MDCK, and OK cells, respectively, in the rest of our study.

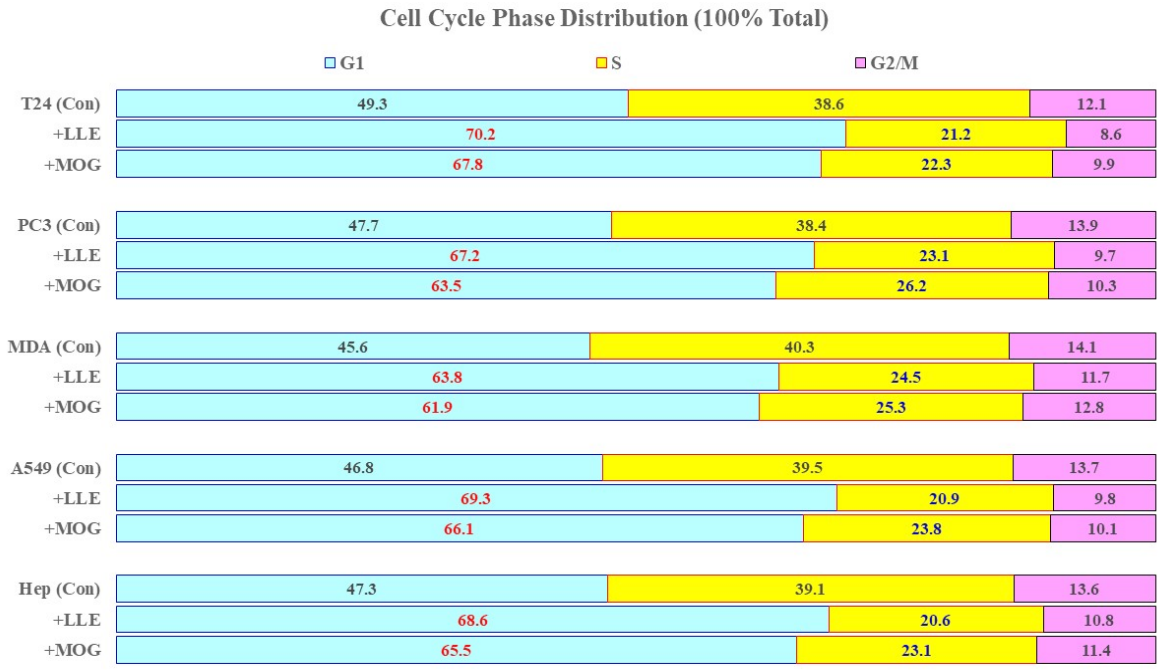


Figure 3: Cell cycle analysis. Five cancer cells were treated with LLE (3 µg/ml) or MOG (2,000 µg/ml) for 72 h and subjected to cell cycle analysis. The cell distribution (%) of each cell cycle phase (G_1, S, and G_2/M) in those cancer cells is illustrated. Although the mean ± SD were calculated for all data obtained from three separate experiments, only the mean values are shown here. The values (%) of G_1 and S phases in all five cancer cells treated with LLE or MOG are statistically ($p < 0.05$) different from those in respective controls.

Possible Antioxidant Activity of LLE and MOG

Now, whether any products (LS, LME, LLE, or MOG) would have antioxidant activity capable of protecting three normal cells from being injured (or killed) by OXS was examined. LLC-PK_1 cells were treated with H_2O_2 (65 µM) alone or with LS (500 µg/ml), LME (500 µg/ml), LLE (3 µg/ml) or MOG (2,000 µg/ml) and cell viability was determined in 72 h. Although cell viability was reduced by ~50% with H_2O_2 in LLC-PK_1 cells, the ~33% and ~25% *increases* in such reduced cell viability were seen with LLE and MOG, respectively (Figure 6A). However, LS and LME had little effects against an OXS attack.

Similarly, the two other experiments, using 60 µM H_2O_2 in MDCK cells and 45 µM H_2O_2 in OK cells, showed nearly the same results. Both LLE and MOG significantly ($p < 0.05$) protected the cells from OXS, whereas LS and LME had little effects (Figures 6B and C). Thus, these results suggest that LLE and MOG appear to have antioxidant activity capable of protecting normal cells from an OXS assault.

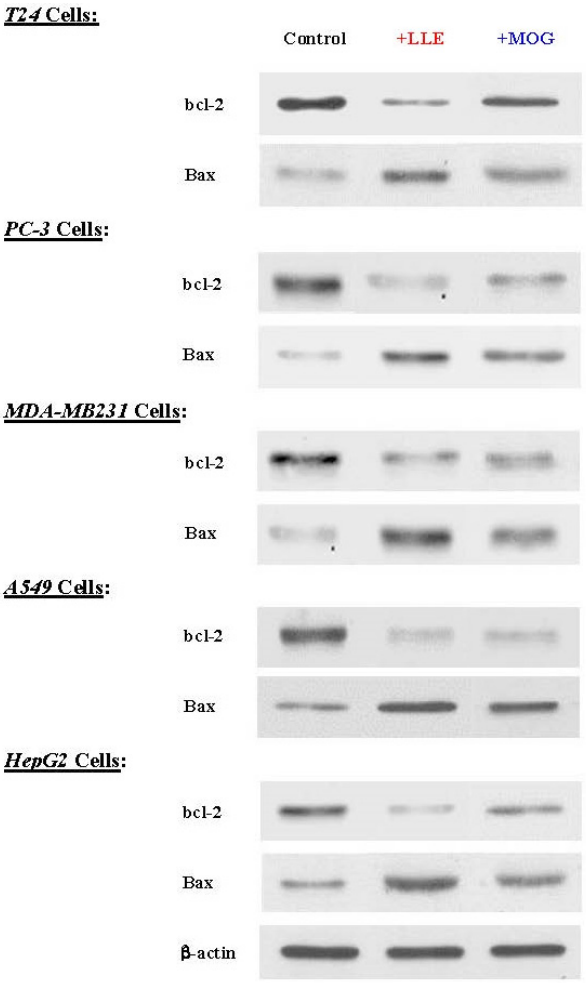


Figure 4: Induction of apoptosis with LLE or MOG. Five cancer cells treated with LLE (3 µg/ml) or MOG (2,000 µg/ml) for 72 h were subjected to Western blot analysis for two apoptotic regulators, bcl-2 and Bax. Autoradiographs of the protein expressions of bcl-2 and Bax in control, LLE-treated, or MOG-treated cells for all five cancer cells are shown. Additionally, β-actin is also included as an internal loading control.

Reduction in Severity of OXS with LLE and MOG

To further confirm antioxidant activity of LLE and MOG, we directly measured how much (H_2O_2-induced) OXS was actually reduced/abolished with LLE or MOG. The severity of OXS can be determined by lipid peroxidation (LPO) assay, measuring the *amount* of malondialdehyde (MDA) formed under OXS – *the more MDA formed, the severer OXS.* It should be noted that both LS and LME were omitted from this study because they had little effects against OXS (Figures 6A-C). Three normal cells were exposed to specified concentrations (65, 60, or 45 µM) of H_2O_2 in the absence or presence of LLE (3 µg/ml) or MOG (2,000 µg/ml) for 6 h. As it is known that OXS usually takes place at the early phase, a 6-h incubation period should be sufficient to properly monitor the severity of OXS. The assay revealed that the amounts of MDA formed under OXS (induced by H_2O_2) were ~3.2-, ~2.8-, and ~3-fold increases (compared to control) in LLC-PK_1, MDCK, and OK cells, respectively (Figure 7). These results are indicative of *severe* OXS exerted on cells; however, such OXS was significantly ($p < 0.05$) reduced by ~30%, ~28%, and ~32% with LLE (3 µg/ml) in LLC-PK_1, MDCK, and OK cells, respectively (Figure 7).

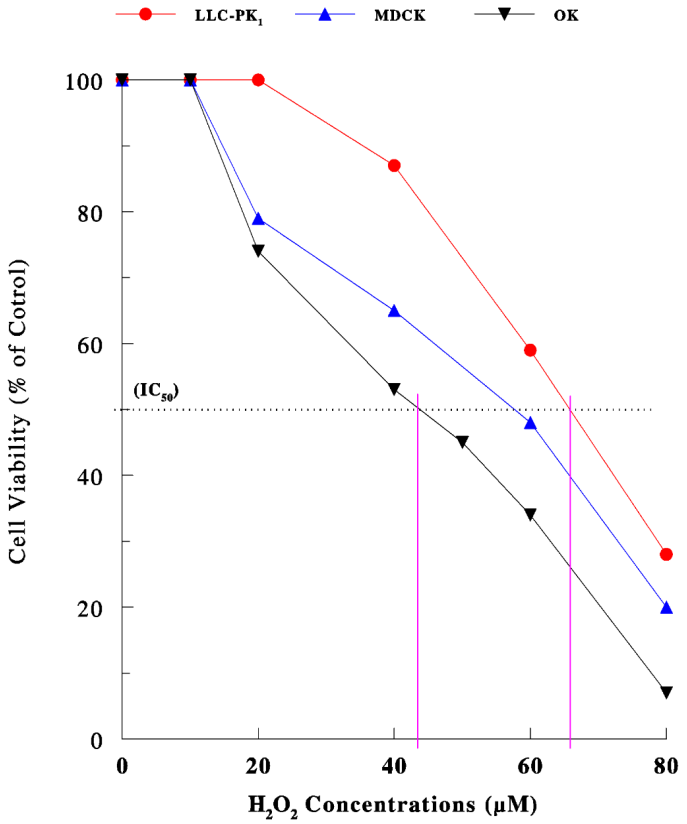
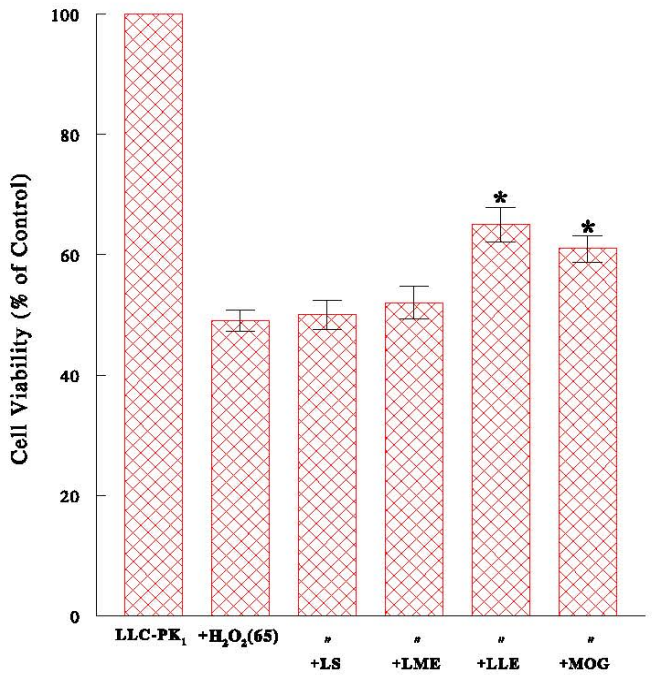
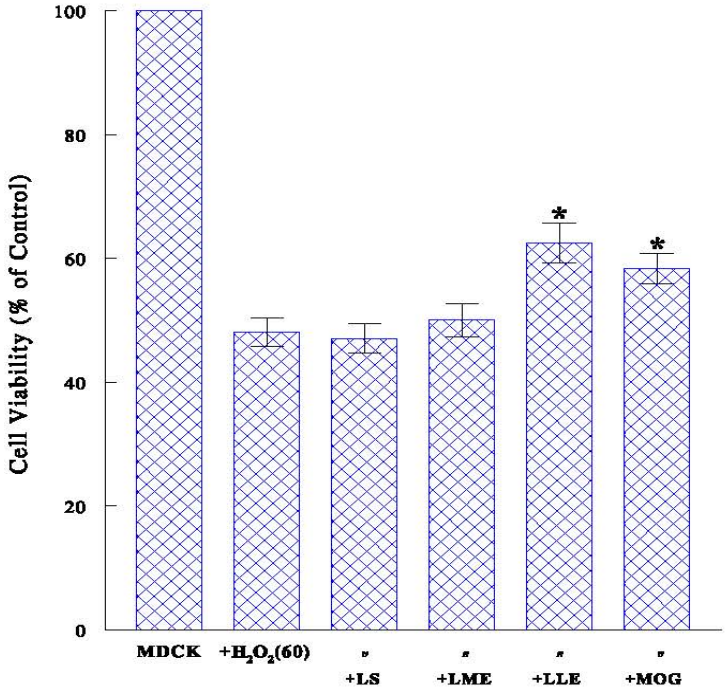


Figure 5: Effects of H_2O_2 on normal cells. Three types of normal kidney cells, LLC-PK_1, MDCK, and OK cells, were treated with the varying concentrations (0-80 µM) of H_2O_2 for 72 h and cell viability was determined by MTT assay. Although the mean ± SD were calculated for all data obtained from three independent experiments, only the mean values (without error bars) are plotted here. The IC_{50} values of H_2O_2 for three cells can be also extrapolated from a graph.



A



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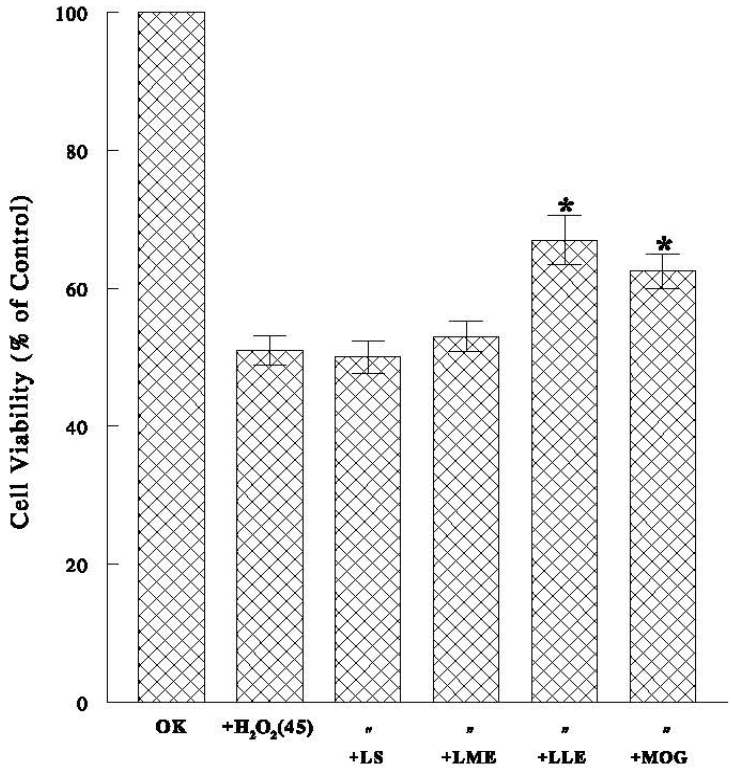

C

Figure 6: Protective effects of four products against H_2O_2 cytotoxicity. LLC-PK_1 **(A)**, MDCK **(B)**, and OK **(C)** cells were treated with 65, 60, and 45 µM of H_2O_2, respectively, in the absence or presence of four products for 72 h. Cell viability was determined and the mean ± SD were calculated for all data from three separate experiments (*$p < 0.05$ compared with H_2O_2 alone).

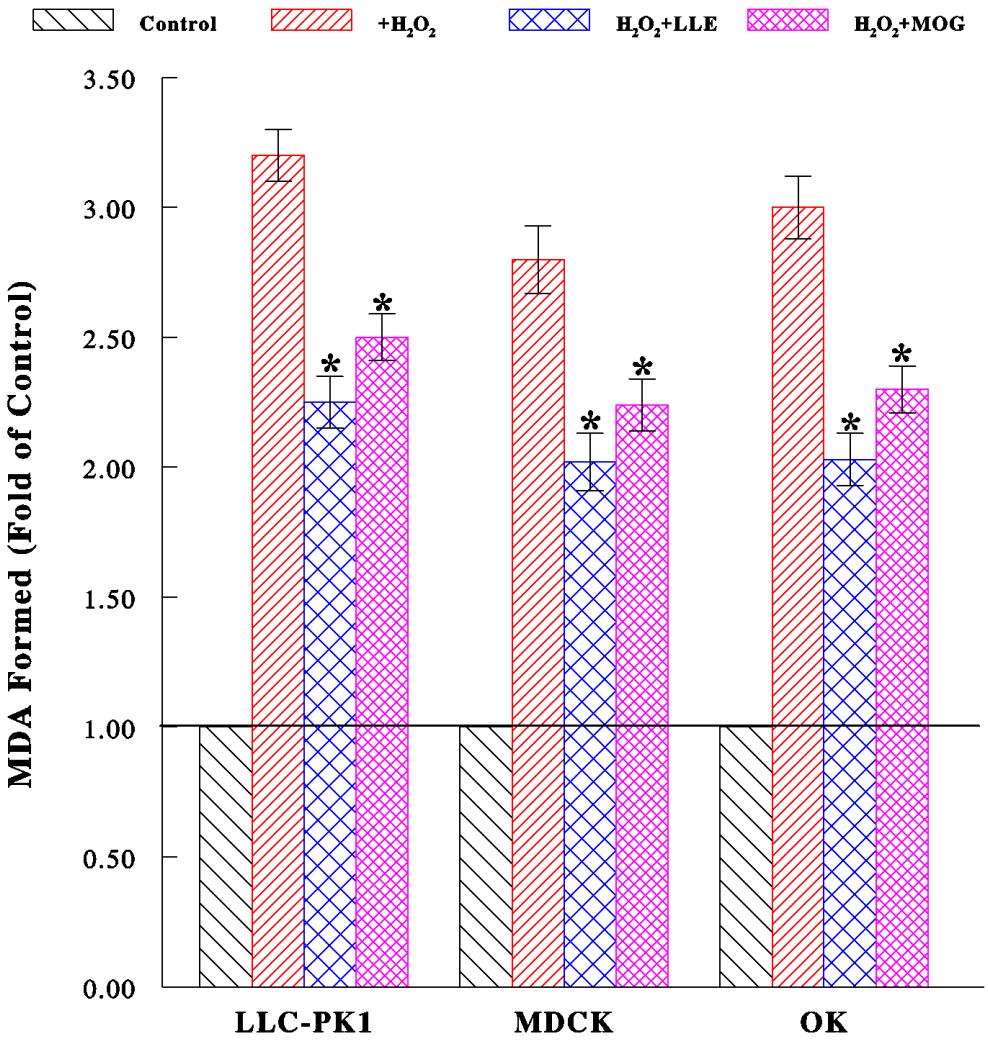

Figure 7: Assessment of severity of OXS exerted on cells. Three normal cells were treated with specified concentrations of H_2O_2 in the absence/presence of LLE (3 µg/ml) or MOG (2,000 µg/ml) for 6 h and subjected to LPO assay to measure the amounts of MDA formed. All data shown are the mean ± SD from three independent experiments (*p <0.05 compared with H_2O_2 alone in respective control cells).

Similarly, the amounts of MDA formed by OXS were also reduced by ~22%, ~20%, and ~23% with MOG (2,000 µg/ml) in LLC-PK_1, MDCK, and OK cells, respectively (Figure 7). Therefore, both LLE and MOG further demonstrate their antioxidant activity capable of significantly reducing H_2O_2-induced OXS, protecting these normal cells.

Discussion

Everyday we are facing the unforeseen health threats, such as cancer, oxidative stress (OXS), various unknown/unidentified diseases etc., so that we are constantly having critical challenges. Particularly, cancer and OXS have something in common – *everybody* is at the risk of getting them. Hence, it was tempting us to find the better way to prevent and/or treat cancer as well as OXS-mediated health issues. We recently came across an interesting natural product with medicinal or pharmacological properties, which could be used in the preventative or therapeutic purpose.

Mogrosides (with several derivatives), isolated from monk fruit, are terpenoid glycosides and found to have several beneficial properties including anticancer and antioxidant activities [17]. Specifically, *mogroside V*-based proprietary products are commercially available as the Lakanto® products (Saraya Co., Ltd.). Since they are easily available and could be used/consumed daily, they would be rather beneficial to people if they indeed have such biological activities. We thus investigated possible anticancer and antioxidant activities of these aspiring products *in vitro*.

Our first study on possible anticancer effect of four Lakanto® products (LS, LME, LLE, and MOG) showed that LLE and MOG had anticancer effect, significantly ($p < 0.05$) reducing cell viability in five different cancer cells (T24, PC-3, MDA-MB231, A549, and HepG2) (Figures 2B and C). However, LS and LME had little effects, indicating that they have no apparent anticancer activity (Figure 2A). These results also suggest that LLE and MOG appear to work *universally* in a non-cancer specific manner, thereby possibly working for a variety of cancers. Regarding the effective

concentrations of LLE and MOG, they are rather different as only 3 μg/ml of LLE and 2,000 μg/ml of (purified) MOG are required to be most effective. These concentrations seem to be somewhat inconsistent, although they are yet calculated from information available in the analytical sheet. *Purified* MOG is a powder material and its (liquid) stock can be accurately prepared for experiments; however, LLE is originally in a *liquid* form and its MOG concentration is purely based on our calculation. Honestly speaking, we cannot rule out the ambiguity if the concentration (3 μg/ml) of MOG in LLE used was what it really was and what other materials or components would have been also present. Nevertheless, it is apparent that LLE does have a significant anticancer effect, although its effective concentration could be somewhat elusive and needs to be adequately determined.

We then explored the anticancer mechanism of LLE and MOG, focusing on cell cycle. Such study showed that treatment of LLE or MOG led to a G_1 cell cycle arrest where cells were arrested or accumulated in the G_1 phase (of cell cycle), due to an inhibition of cells entering to the next S phase (Figure 3). As a result, the cell population or number in the G_1 phase *increases* while that in the S phase *decreases*, resulting in an accumulation of cells in the G_1 phase, i.e. a G_1 cell cycle arrest [30]. All five cancer cells experienced this cell cycle arrest with LLE and MOG, at least in part accounting for the resulting reduction in cell viability. As this cell cycle is the key regulatory mechanism for cell division, proliferation, and development, its inhibition or interruption by drugs, chemicals, biologicals etc. would lead to the cessation of cell growth and even cell death. Thus, LLE and MOG may primarily target the G_1 phase, eventually inhibiting the cancer cell growth.
Since it is worthwhile addressing what the fate of cancer cells would be with LLE and MOG, we examined if they might eventually undergo apoptosis that often offers clinical implications. Apoptosis or programmed cell death is also considered as "cell suicide", as opposed to necrosis or "cell murder" caused typically by chemotherapy [32]. In short, apoptosis is a highly organized biochemical death process *without* rupturing cells to release cytotoxic materials that would cause secondary injury or inflammation to the surrounding cells/tissues (manifesting "side effects") [32].

During apoptosis, only (cancer) cells determined to commit suicide would quietly die out without creating any side effects, which often result from chemotherapy that randomly kills or murders cancer as well as normal cells with releasing toxic cytoplasmic contents, resulting in secondary injury or inflammation. Hence, as apoptosis may cause few side effects unlike necrosis (through chemotherapy), any regimens ultimately inducing apoptosis (in cancer cells) are safer and more suitable to those cancer patients. Our study then revealed that all cancer cells treated with LLE or MOG underwent apoptosis, indicated by the down-regulation of (anti-apoptotic) bcl-2 and the up-regulation of (pro-apoptotic) Bax (Figure 4). Thus, both LLE and MOG may act as an apoptotic inducer, capable of inducing apoptosis in cancer cells, and could be potentially used clinically.

We next studied if any of four products might have *antioxidant* activity against OXS. In this study, we employed the three different normal kidney cells and examined if any products could protect these cells from being injured or killed by H_2O_2-induced OXS. As expected, OXS was cytotoxic and reduced cell viability in all cells (Figure 5), but LLE and MOG reduced OXS to a certain extent, sustaining *higher* cell viability (than that reduced by OXS) owing to their antioxidant activity (Figures 6A-C). Additionally, the actual severity of OXS exerted on cells were determined by the LPO assay, measuring the amount of MDA formed under OXS. MDA is a product yielded from peroxidation of polyunsaturated fatty acids in the plasma membrane, due to OXS attack [28]. As the amounts of MDA formed would fairly reflect the severity of OXS, a nearly 3-fold increase in MDA formation by (H_2O_2-induced) OXS is indicative of severe OXS exerted on three normal cells. However, LLE and MOG significantly reduced MDA formation and diminished the severity of OXS too (Figure 7). Thus, LLE and MOG, not LS and LME, may have antioxidant activity to reduce OXS, protecting normal cells from a detrimental oxidative assault.

After all, it is promising that LLE and MOG appear to have both anticancer and antioxidant activities, but what does that really mean? With anticancer activity, as they can stop cancer cell growth and subsequently reduce cell viability, they could be used in the cancer treatment. On the other hand, as antioxidant activity may generally protect our body or cells from harmful OXS or even OXS-induced cancer development, they could be more likely used in the cancer *prevention*. However, the same question is always raised in this kind of study – although a drug or agent has anticancer activity to inhibit cancer cell growth, what would happen to healthy/normal cells? If the growth of normal cells were also equally inhibited by such a drug/agent, it appears to randomly attack cancer and normal cells and will not be useful in the therapeutic purpose. Our study then showed that LLE and MOG reduced cell growth/viability in (five different) *cancer* cells but had little effects on three *normal* cells. Thus, these products may act as anticancer agents to inhibit the cancer growth and as antioxidant agents to prevent the cancer development. Nevertheless, this is yet the *in vitro* study and more studies are certainly required for confirming their actual efficacy, dosages, and safety in animals (*in vivo*). Our next study will use tumor-bearing mice to find if LLE or MOG may actually demonstrate their anticancer/antitumor activity in mice. Moreover, antioxidant activity will be also assessed in a rat model – whether renal ischemia/reperfusion injury (RIRI) induced in rats could be alleviated or prevented with LLE or MOG will be examined because RIRI is known to be primarily attributed to OXS. Further studies are thus warranted.

Conclusions

In this study, two of four Lakanto® products tested, LLE and MOG, demonstrated their anticancer and antioxidant activities. They are capable of reducing cell viability of various cancer cells and inducing apoptosis, while they can also protect normal cells from oxidative stress-mediated cell injury and cell death by reducing/abolishing oxidative stress. Therefore, these products (and other

unverified similar products) may have clinical implications in the cancer prevention/treatment.

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