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Chemosensitizing Effect of Monk Fruit Extract on Human Bladder Cancer Cells

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Abstract

The outcomes of chemotherapy have been unsatisfactory with the palpable side effects. We hypothesized that *natural products* might help improve chemotherapy with few side effects. Recently, we came across the bioactive extracts of monk fruit (*Siraitia grosvenori*) with anticancer activity. We then investigated if these extracts might have chemosensitizing effect to improve the efficacy of drugs clinically used today. Four different drugs, cisplatin (CPL), carboplatin (CBL), mitomycin C (MMC), and gemcitabine (GEM), were used in this study. Human bladder cancer T24 cells were treated with each drug itself or drug combined with either LLE or MOG (two types of monk fruit extracts). Cell viability was determined to assess anticancer effect and also explored the anticancer mechanism of such combinations, focusing on the status of glycolysis, cell cycle, and chromatin structure. Cell viability test showed that all drugs had anticancer activity, reducing cell viability, but only CPL showed the enhanced anticancer effect when combined with LLE (not with MOG). The rest of three drugs had no such effects with LLE or MOG. The *CPL/LLE combination* was found to disrupt glycolysis, by inhibiting hexokinase activity, resulted in the decreased ATP synthesis. This combination also blocked the cell cycle progression, due to a G_1 cell cycle arrest. Moreover, the two epigenetic regulators, DNA methyltransferase and histone deacetylase, were inactivated with the combination, indicating chromatin modifications. Ultimately, these treated cells were found to undergo apoptosis. In conclusion, anticancer activity of CPL can be significantly enhanced with LLE. This chemosensitizing effect is attributed to the glycolysis inhibition, a G_1 cell cycle arrest, and chromatin modifications, ultimately leading to apoptosis. Thus, certain *natural products* such as LLE could be used as an adjuvant agent in current chemotherapy, improving the drug efficacy but minimizing side effects.

Keywords

Monk Fruit, Anticancer, Bladder Cancer, Chemosensitization, Lakanto

1. Introduction

Bladder cancer is a growing epidemic and a major global health challenge with an annual estimated 991,000 new cases and 397,000 deaths worldwide by 2040 [1]. In the United States alone, approximately 83,000 new cases were diagnosed and nearly 17,000 patients died from bladder cancer in 2023 [2]. Over the past 20 years, the 5-year survival rates of bladder cancer have been 33% and 5% for locally advanced and metastatic disease, respectively [3]. This is a serious challenge to urgently develop/establish the *better* bladder cancer prevention and treatment.

Currently, intravesical administration of bacillus Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis*, is the most effective immunotherapy available for high-grade and recurrent superficial bladder cancer and carcinoma *in situ* (CIS) [4]. Side effects of this therapy are yet common, including cystitis, hematuria, and other adverse effects (fever, allergic reactions, sepsis etc.) [4]. Hence, these disadvantages considerably limit its use in clinical practice [4] [5].

Meanwhile, chemotherapy using a variety of cytotoxic drugs, including cisplatin, mitomycin C, gemcitabine, 5-flurouracil, cyclophosphamide, doxorubicin etc., and their multiple combinations are widely used in patients with various cancers, including bladder cancer [6]. However, palpable side effects of chemotherapy are a major issue and its efficacy is also of limited duration and no significant advantage in survival has been found in patients [6] [7].

Despite such inevitable drawbacks, chemotherapy is yet a mainstream therapeutic option, which is widely and routinely used in various cancer patients [6]. It is thus important and beneficial if the efficacy of chemotherapeutic drugs is somehow improved, while side effects are significantly minimized or alleviated. Interestingly, it has been often reported that the combination of drugs and certain *natural agents/products* with few side effects demonstrated the improved anticancer effects against cancer cells (*i.e.*, chemosensitization) [8] [9] [10]. Thus, we were interested in *natural products* (with few side effects), which could be used as an adjuvant agent for chemotherapy.

We have been working on various natural products over a decade and recently came across the bioactive extracts of monk fruit (*Siraitia grosvenori*) [11], which were commercially available as Lakanto® (LKT). All LKT products are the proprietary products that have been developed by the Japanese company (Saraya Co., Ltd., Osaka, Japan). Our recent study [12] using four LKT products showed that the two products called LLE and MOG had significant anticancer effect against five different cancers including bladder cancer. Briefly, active components of LKT products are *mogrosides*, which have been widely used for commercial dietary products as the US Food and Drug Administration (FDA) had approved them for Generally Recognized As Safe (GRAS) [13]. In addition, a number of scientific/medical studies also revealed pharmacological properties of mogrosides, including anticancer/antitumor, anticarcinogenic, antioxidant, anti-diabetic activities etc. [14] [15] [16] [17] [18]. However, prior to our recent

study, few anticancer effects of LKT products have been fully studied.

It was then tempting us to examine if the two LKT products, LLE and MOG, could enhance or improve anticancer activity of chemotherapeutic drugs clinically used (*i.e.*, chemosensitization). As these two products are natural products with few side effects, it is plausible that they may enhance anticancer activity of drugs while minimizing side effects.

Accordingly, we investigated if LLE and/or MOG might have chemosensitizing effect to improve the drug efficacy on bladder cancer cells. We also explored to understand the anticancer mechanism of such combinations, focusing on glycolysis, cell cycle, chromatin structure, and apoptosis. More details are described and the interesting findings are also discussed herein.

2. Materials and Methods

2.1. Cell Culture

Human bladder cancer T24 cells (Grade 3) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). They were cultured in RPMI 1640 medium (Corning, Corning, NY), supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) in an incubator at 37˚C. For experiments, they were seeded at the initial cell density of 2×10^5 cells/ml in the 6-well plates or T-75 flasks, and the dose-dependent effects of four drugs clinically used today, cisplatin (CPL), carboplatin (CBL), mitomycin C (MMC), or gemcitabine (GEM), were examined in 72 h. Each drug was also combined with either LLE or MOG (Saraya Co., Ltd.) to assess a possible chemosensitizing effect. At 72 h, cell viability, *i.e.*, the number of viable cells, was determined by MTT assay described below.

2.2. MTT Assay (Cell Viability Test)

We define anticancer effect by determining *what %* of T24 cells is still "alive" after the drug treatments (with LLE or MOG). Assays were performed essentially following the vendor's protocol (Sigma-Aldrich, St. Louis, MO). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) reagent (1 mg/ml) was added to each well in the 6-well plate, followed by 3-h incubation at 37˚C. After discarding MTT reagent, 1 ml each of dimethyl sulfoxide was added to the plate to dissolve formazan precipitates (purple color), which was then read at 595 nm on a microplate reader. Cell viability was expressed by the percent (%) of viable cells relative to the control (untreated) reading (100%). As a result, the *higher* cell viability *reduction* (*i.e.*, the lower cell viability) indicates the *greater* anticancer effect.

2.3. Assessment of Glycolysis Inhibition

Hexokinase (HK) Assay

Hexokinase (HK) activity was determined using HK Colorimetric Assay Kit (BioVision, Waltham, MA) following the manufacturer's protocol. Cell lysates

(20 μg per sample) and NADH standards were prepared in the 96-well plate and the reaction was started by the addition of reaction mixture (containing substrate). The plate was placed in a microplate reader and the absorbance (OD) changes with time were monitored at 450 nm for 20 min with 5-min intervals. All readings were calculated using references (NADH standards), and HK activity was expressed by the % of sample readings relative to the controls (100%).

Assay for Cellular ATP Level

Cellular ATP level was determined using ATP Colorimetric Assay Kit (BioVision) following vendor's protocol. Cells (2×10^5 cells/ml) in the 6-well plate were first lysed in ATP assay buffer, deproteinized with $HClO_4$, and neutralized with KOH. Cell lysates (50 μl per sample) and ATP standards were prepared in the 96-well plate and the reaction was started by the addition of reaction mixture. The plate was then incubated at room temperature for 30 min in the dark. Absorbance at 570 nm was read on a microplate reader and ATP contents in samples were calculated by referring to the readings of ATP standards. The ATP level was then expressed by the % of ATP amounts in samples relative to the controls (100%).

2.4. Cell Cycle Analysis

T24 cells treated with given conditions for 72 h were harvested and subjected to cell cycle analysis. Cells (~1×10^6 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 (G_1, S, and G_2/M).

2.5. Assays for DNA Methyltransferase (DNMT) and Histone Deacetylase (HDAC) Activities

Activities of DNMT and HDAC were determined essentially following the vender's protocol (EpiGentek, Farmingdale, NY). Control and agent-treated T24 cells were harvested at 72 h and nuclear extracts were prepared using EpiQuik Nuclear Extraction Kit (EpiGentek). DNMT assay was performed on nuclear extracts using EpiQuik DNMT Activity/Inhibition Colorimetric Assay Kit (EpiGentek) following the given protocol. Similarly, HDAC assay was performed on nuclear extracts above using EpiQuik HDAC Activity/Inhibition Colorimetric Assay Kit (EpiGentek) as well. DNMT and HDAC activities were then calculated and expressed by percent (%) relative to controls (100%).

2.6. Western Blot Analysis

Cell lysates were first obtained from control and agents-treated cells by 3 cycles of freeze-thaw in liquid nitrogen, followed by centrifugation at 13,000 rpm for 10 min at 4˚C. An equal amount of cell lysates (10 μg) was resolved by 10% SDS-

PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred to a nitrocellulose membrane (blot), which was then incubated with 5% milk overnight at 4˚C. The blot was first incubated for 90 min with the primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) against bcl-2 and Bax, followed by incubation for 30 min with the appropriate secondary antibody conjugates (Santa Cruz Biotechnology). The specific immunoreactive proteins (bcl-2 and Bax) were then detected by chemiluminescence (Seracare, Milford, MA) on an X-ray film (autoradiography).

2.7. 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.

3. Results

3.1. Dose-Dependent Effects of Four Drugs on T24 Cell Viability

T24 cells were treated with varying concentrations of four drugs, CPL (0 - 500 nM), CBL (0 - 10 μM), MMC (0 - 500 nM), or GEM (0 - 100 nM), for 72 h and cell viability was determined using MTT assay. CPL ≥ 100 nM led to a significant cell viability reduction with the IC_{50} of ~180 nM, indicating anticancer effect (**Figure 1(a)**). Similarly, CBL ≥ 3 μM also resulted in a significant cell viability reduction with the IC_{50} of ~5 μM (**Figure 1(b)**). MMC ≥ 100 nM showed a significant anticancer effect with the IC_{50} of ~150 nM (**Figure 1(c)**), while GEM ≥ 20 nM demonstrated a similar effect with the IC_{50} of ~40 nM (**Figure 1(d)**). Therefore, all four drugs have potent anticancer activity, capable of significantly reducing cell viability of T24 cells. Such effective concentrations of drugs were also found to be relatively *low* in the range of nM to low μM.

3.2. Possible Chemosensitizing Effect of LLE or MOG on Drugs

Although all four drugs have anticancer activity, whether such effective concentrations would be physiologically achievable and may yet cause side effects in actual patients is uncertain. It is then conceivable and practical if we can find the way to *lower* drug concentrations but *improve* the efficacy. We examined if the two LKT products, *LLE* and *MOG*, might help enhance or improve anticancer activity of drugs (*i.e.*, chemosensitization).The *ineffective* or *low* concentration of each drug (**Figures 1(a)-(d)**) was combined with LLE or MOG as follows: CPL (10 nM), CBL (1 μM), MMC (50 nM) or GEM (10 nM) combined with LLE (1 μg/ml) or MOG (1000 μg/ml). After T24 cells were treated with these combinations for 72 h, cell viability was determined by MTT assay. The results show that only one of four drugs, CPL, showed a significant reduction in T24 cell viability when combined with LLE, not with MOG (**Figure 2(a)**). The rest of three drugs showed no such improvements with LLE or MOG (**Figures 2(b)-(d)**).

Although the MMC/LLE combination yet looked to display an increased cell viability reduction (Figure 2(c)), it was not statistically significant (p = 0.06). Thus, only LLE appears to be capable of significantly potentiating CPL, demonstrating its chemosensitizing effect. Since MOG failed to demonstrate such an effect, it was omitted from the rest of our study.

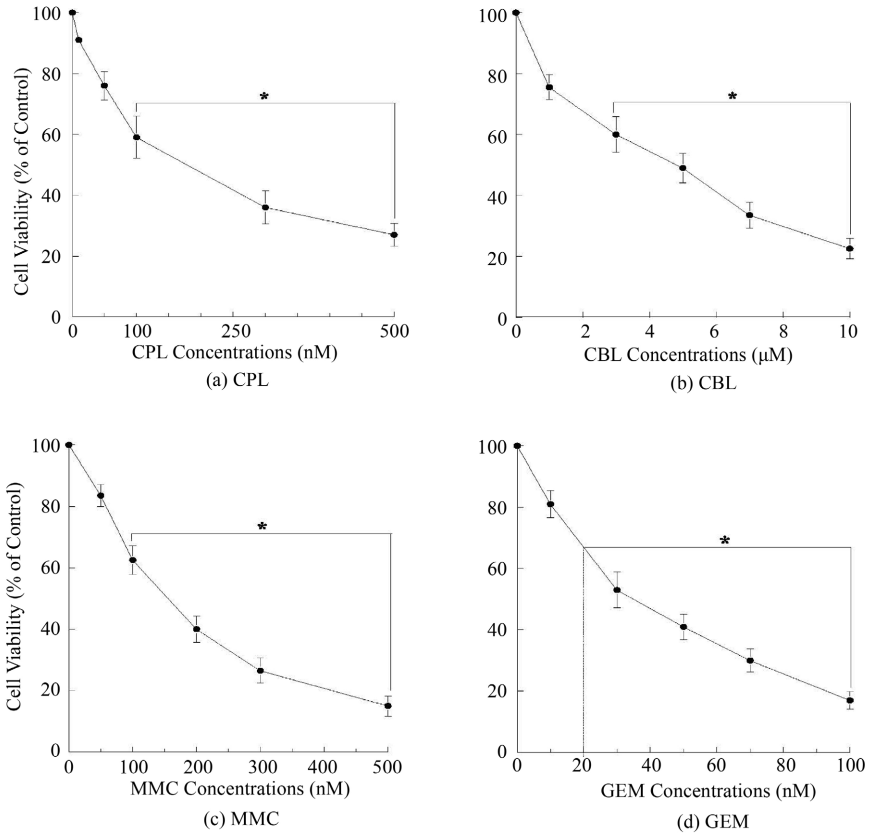
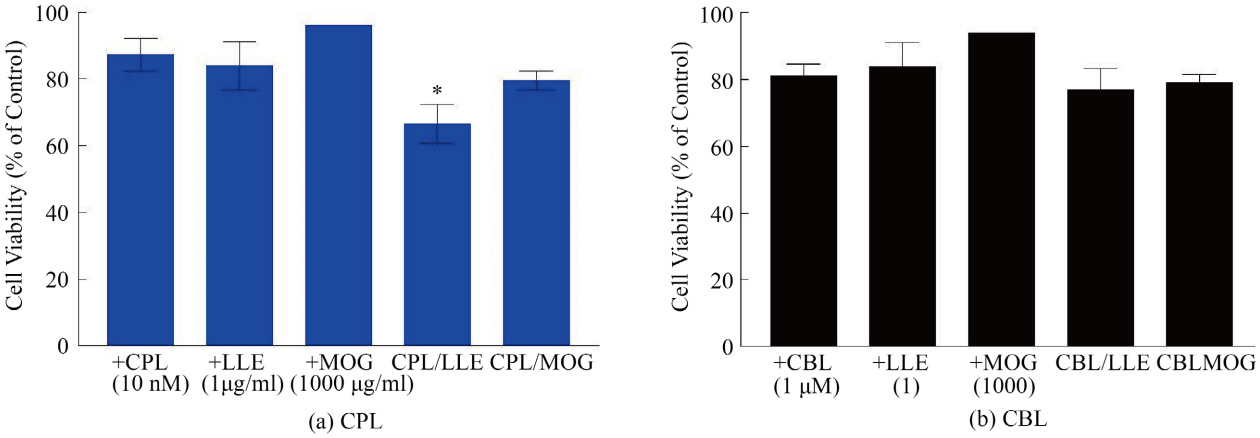


Figure 1. Dose-dependent effect of four drugs, CPL (a), CBL (b), MMC (c), and GEM (d), on cell viability. T24 cells were cultured with varying concentrations of each drug and cell viability was assessed in 72 h by MTT assay. Cell viability was expressed by the percent (%) of viable cells relative to controls (100%). The data are mean ± SD (standard deviation) from three separate experiments (*p < 0.05 compared with control).



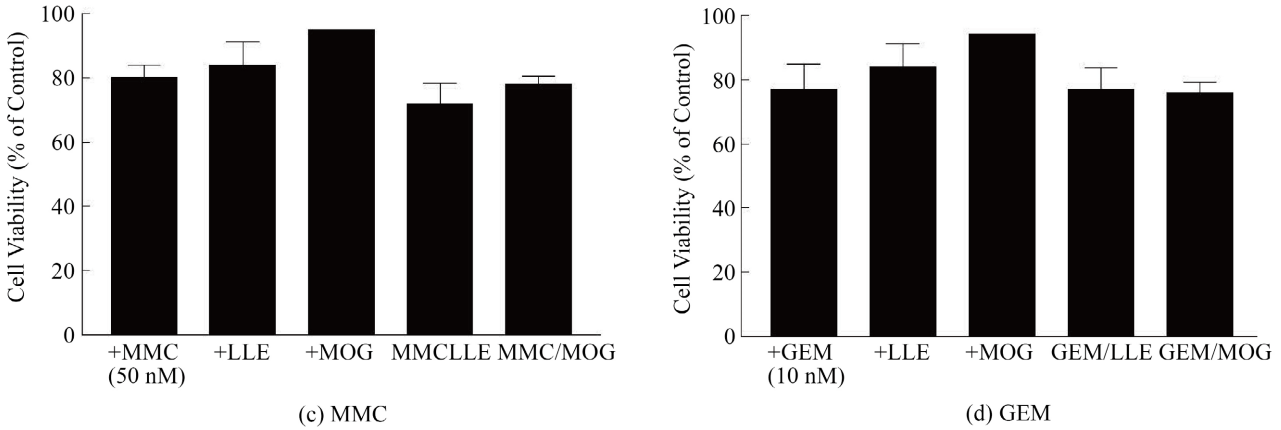


Figure 2. Enhanced anticancer effect of four drugs combined with LLE/MOG. Cells were treated with four different combinations, CPL-LLE/MOG (a), CBL-LLE/MOG (b), MMC-LLE/MOG (c), or GEM-LLE/MOG (d), for 72 h and cell viability was determined by MTT assay. All data are mean ± SD from three independent experiments but error bars are omitted (*$p < 0.05$ compared with respective drug).

3.3. Effects of Drug/LLE Combinations on Glycolysis

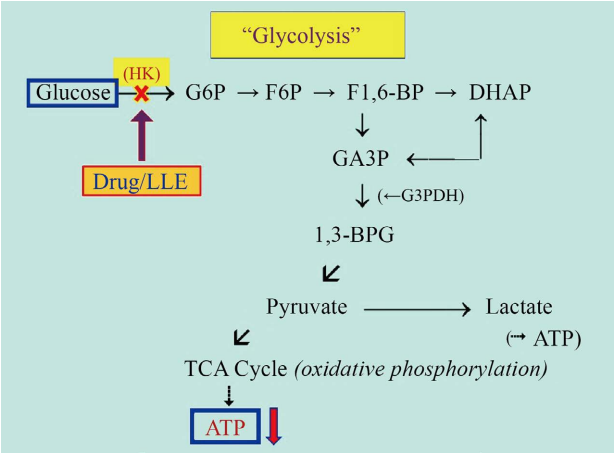


Diagram of Glycolytic Pathway

Although at least CPL seems to respond to LLE, exhibiting increased anticancer activity, it is important to understand how CPL is potentiated with LLE or what the anticancer mechanism might be working. Only the CPL/LLE combination would more likely show significant effects on cellular parameters, but the rest of drugs (CBL, MMC, and GEM) were also tested for comparison. First of all, we examined possible effects of drug/LLE combinations on glycolysis, which was the vital metabolic process required for cell proliferation and survival [19] [20]. As hexokinase (HK) is one of key glycolytic enzymes involved in the irreversible committed step in glycolysis (*See* a diagram above) [21], its inactivation or inhibition would result in a disruption of glycolysis, which in turn leads to the reduction in ATP synthesis (a diagram). T24 cells were then treated with all four combinations, CPL/LLE, CBL/LLE, MMC/LLE, or GEM/LLE, for 72 h and subjected to HK activity and ATP synthesis assays. We found that only the CPL/LLE combination led to a significant (27%) decrease or inactivation of HK,

whereas no such HK inactivation was seen with other combinations (Figure 3(a)). Once again, inactivation of HK with the MMC/LLE combination seemed to be possible, but it was not statistically significant (p = 0.06). Moreover, the amount or level of ATP synthesized/yielded was also significantly (~30%) reduced with the CPL/LLE combination, whereas no such reduction was seen with the other combinations (Figure 3(b)). Therefore, these findings suggest that the glycolytic pathway is critically disrupted or inhibited with the CPL/LLE combination, resulting in the incompletion of glycolysis.

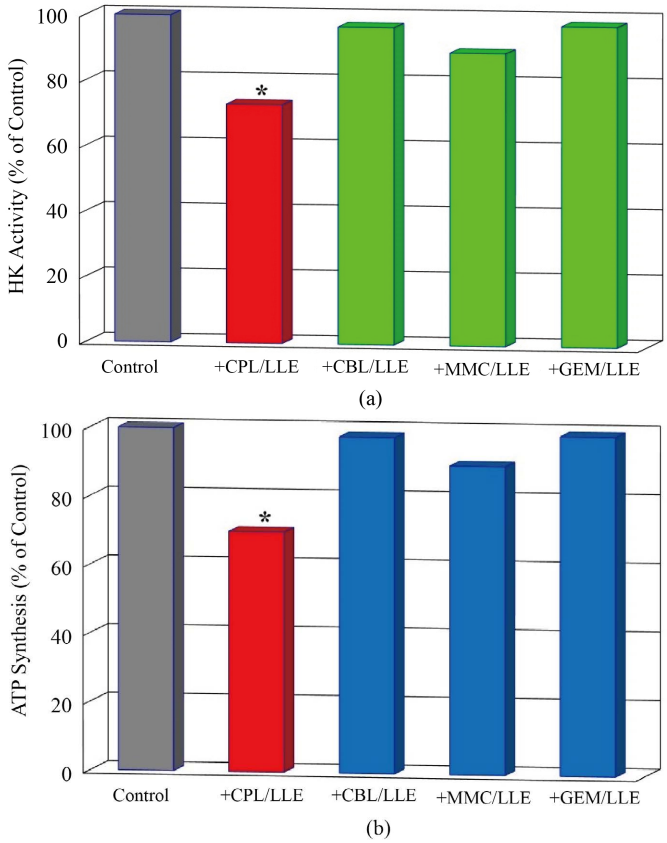


Figure 3. Effects of four different "drug/LLE" combinations on hexokinase (HK) activity (a) or ATP synthesis (b). Cells were treated with four different drugs (CPL, CBL, MMC, or GEM) combined with LLE for 72 h and cell viability was determined. All data are mean ± SD from three separate experiments but error bars are omitted (*p < 0.05 compared with control).

3.4. Effects of Drug/LLE Combination on Cell Cycle

Besides glycolysis required for cellular energy supply, another key cellular event required for continuing cell growth is to successfully go through cell cycle. Any interruption or inhibition of the cell cycle progression/transition could result in growth cessation and cell death [22]. T24 cells were treated with four combinations for 72 h and subjected to cell cycle analysis, which would determine the % of cells distributed in the different cell cycle phases (G_1, S, and G_2/M). Analysis revealed that only the CPL/LLE combination resulted in the *increase* in the

G_1-phase cell population from 60.1% (CPL alone) to 70.1% ($p < 0.05$), while the S-phase population *decreased* from 27.3% (CPL alone) to 19.2% ($p < 0.05$) (**Figure 4(a)**). However, no such apparent changes were detected with three other combinations (similar to controls) (**Figure 4(a)**, **Figure 4(b)**). This accumulation of cells in the G_1 phase is known as a G_1 cell cycle arrest [23], subsequently leading to the growth cessation or cell death, which may also account for such improved anticancer effect.

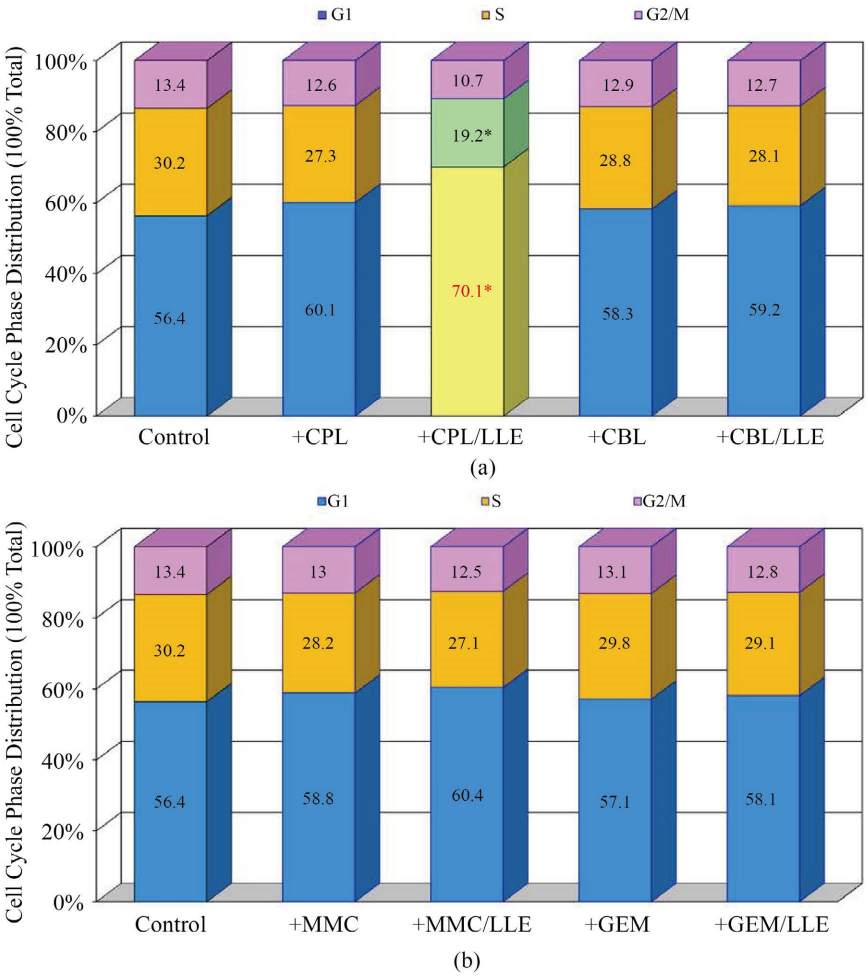


Figure 4. Cell cycle analysis. After T24 cells were treated with four different "drug/LLE" combinations for 72 h, they were subjected to cell cycle analysis to assess any effects on cell cycle. Cell cycle distributions (%) at the G_1, S, and G_2/M phases with each treatment ((a) and (b)) were then plotted by the 100% stocked bars. Although the SD values were calculated, no error bars are shown (*$p < 0.05$ compared with control).

3.5. Chromatin Modifications Induced with Drug/LLE Combinations

Cell cycle has been known to be linked to alterations in chromatin structure, which would substantially regulate cell division and cell growth [24]. Such changes can be controlled by the two key epigenetic regulators, DNA methyltransferase (DNMT) and histone deacetylase (HDAC) [25]. This possibility was

then tested. T24 cells were treated with four combinations for 72 h and subjected to assays for DNMT and HDAC activities. Such assays showed that compared to controls, both DNMT and HDAC activities were significantly (35% - 40%) lost with the CPL/LLE combination (Figure 5(a), Figure 5(b)), but no changes were seen with the three other combinations (data not shown). Since the decrease/loss in both DNMT and HDAC is indicative of changes in chromatin structure, the CPL/LLE combination may result in chromatin modifications.

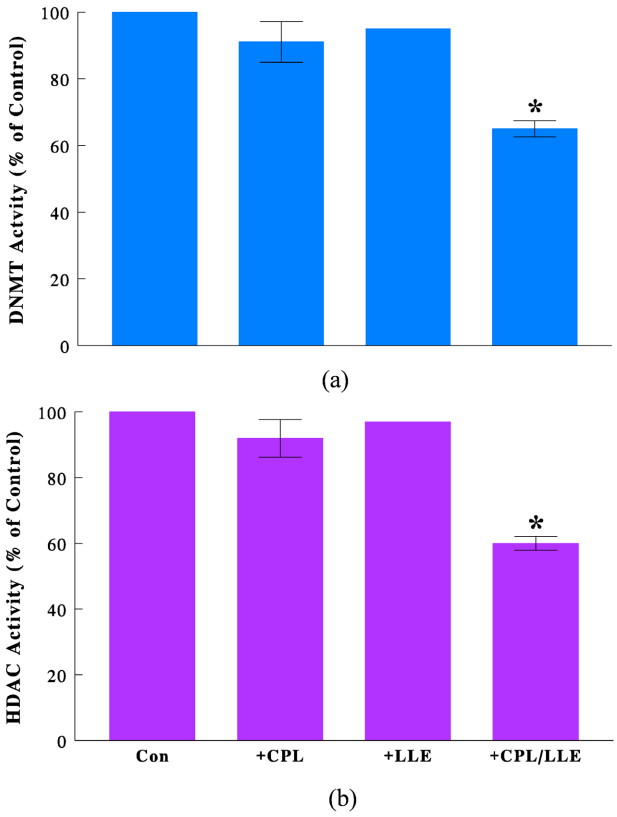


Figure 5. Effects of "drug/LLE" combinations on chromatin structure. Cells treated with four different combinations for 72 h were assayed for activities of two epigenetic regulators, DNMT (a) and HDAC (b). Activities were calculated as mean ± SD from three separate experiments and expressed by the % relative to control (100%). Only the results of CPL/LLE combination with significant changes (*$p < 0.05$ compared with control) are shown here because little effects/changes were seen with other combinations (data not shown).

3.6. Induction of Apoptosis with Drug/LLE Combinations

Lastly, we wondered *what* would ultimately happen to those treated cells? Or, what is the fate of cells? We then examined if they might undergo apoptosis (programmed cell death) because of clinical implications. T24 cells were treated with four combinations and subjected to Western blot analysis, assessing the protein expression of two key apoptotic regulators, bcl-2 an Bax [26]. Analysis revealed that compared to the expressions of bcl-2 and Bax in control, cells treated with the CPL/LLE combination led to the down-regulation (decrease) of

bcl-2 expression but the up-regulation (increase) of Bax (**Figure 6**). However, no such changes were observed with any other combinations as the protein expressions remained as the same as controls (**Figure 6**). Since bcl-2 is known as an anti-apoptotic regulator while Bax is a pro-apoptotic regulator [26], the expression pattern of *decreased* bcl-2 concomitant with *increased* Bax indicates induction of apoptosis. Therefore, T24 cells will ultimately follow the apoptotic pathway when treated with the CPL/LLE combination. It is also plausible that LLE could be considered as an apoptotic inducer when combined with CPL. Moreover, this CPL/LLE-induced apoptosis may have clinical implications, which will be discussed later.

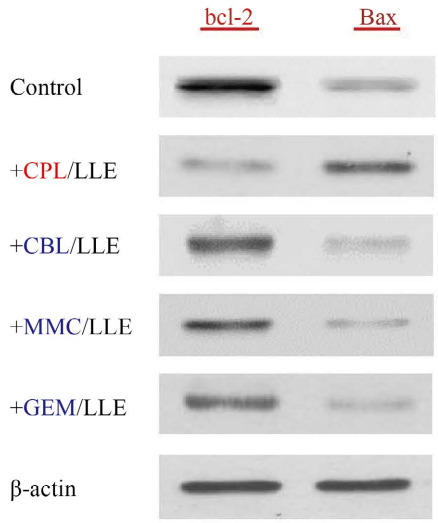


Figure 6. Induction of apoptosis. After cells were treated with four different combinations for 72 h, the expressions of two apoptotic regulators, bcl-2 and Bax, were analyzed on Western blots. Autoradiographs of these regulators in four different conditions are shown for comparison. Beta-actin was also run as an internal control of protein load.

4. Discussion

To improve the efficacy of chemotherapy with minimizing its related side effects, we studied if two LKT products, LLE and MOG, would potentiate four chemotherapeutic drugs, CPL, CBL, MMC, and GEM, which are clinically used in patients with bladder cancer today. We found that all four drugs principally had anticancer effect with varying IC_{50} values (**Figure 1**).

Both LLE and MOG are *natural* extracts/products commercially available as Lakanto® (LKT) products with anticancer activity but few side effects. We thus examined if these products might be able to improve anticancer effect of four drugs (while minimizing side effects) when combined. Such study revealed that anticancer activity of only CPL, not CBL, MMC, and GEM, was found to be significantly potentiated when combined with LLE, but not with MOG (**Figure 2**). We then explored *how* such a specific combination might be able to improve an-

ticancer activity (*i.e.*, anticancer mechanism).

When the possible effect on glycolysis was first examined, the CPL/LLE combination specifically inactivated HK, one of the key glycolytic enzymes, at the early phase of glycolysis [21]. Due to this inactivation, the rest of glycolytic pathway was halted, leading to the significant reduction in the amount of ATP synthesized/yielded, a final product of glycolysis (**Figure 3**). Thus, this glycolysis inhibition would lead to adverse consequences such as growth cessation, cellular dysfunction, and even cell death, at least accounting for its enhanced anticancer activity.

We then examined the status of cell cycle, which was particularly important to be involved in cell proliferation. Such study showed that a G_1 cell cycle arrest [23] was induced only with the CPL/LLE combination (**Figure 4(a)**), preventing the completion of cell cycle progression (from the G_1 to the M phase). It is thus plausible that such a cell cycle arrest will eventually lead to the cell viability reduction as well.

As it was possible that a cell cycle arrest could also affect the DNA configuration, particularly the chromatin structure, the status of two epigenetic regulators, DNMT and HDAC [25], was then assessed. The CPL/LLE combination was found to significantly inactivate both DNMT and HDAC (**Figure 5(a)**, **Figure 5(b)**), indicating chromatin modifications. Thus, the CPL/LLE combination appears to directly affect the chromatin structure, eventually leading to the growth cessation and cell viability reduction.

We lastly examined what the fate of cells treated with the CPL/LLE combination – will they ultimately undergo apoptosis? Such analysis suggested that cells treated with the CPL/LLE combination would most likely follow the apoptotic pathway as indicated by the modulated expressions of two apoptotic regulators (bcl-2 and Bax) [26] (**Figure 6**).

What is the significance of such induction of apoptosis? It could have clinical relevance—any drugs, agents, biologicals etc. capable of inducing apoptosis may *not* have severe side effects when they are given to cancer patients. Hence, it was rather important to address if the CPL/LLE combination would induce apoptosis in bladder cancer cells. As shown here, it did induce apoptosis, implying that combining drug (CPL) and non-drug material (LLE) may apparently improve the drug efficacy while minimizing potential side effects. Therefore, it is plausible that an apoptosis inducer as well as a chemosensitizing agent (e.g., LLE) could be used as an adjuvant agent in the current chemotherapy protocol.

In fact, the medicinal aspects of various natural products/agents have been receiving more public attention, and their sales and use indeed continue to increase worldwide [27]. Those include herbs, mushrooms, flowers, fruits, plant seeds, sea weeds, algae, tea, bark, shark cartilage etc., and they are alternatively used as chemopreventive and adjunctive agents in a variety of cancer cases [28]. Hence, it is even more important to actively and extensively seek and unveil unidentified hidden apoptosis inducers (of natural products).

Last of all, it is rather peculiar or interesting to find out why only CPL, not all four drugs, seems to respond well to LLE to enhance anticancer activity. Although more studies are required for a clear elucidation, it is yet understandable that *mode of action* of four drugs are somewhat different, probably accounting for the discrepancy in a response to LLE. Nevertheless, further investigations will clarify it all and should be attempted. In addition, as the finding in this study seems to be quite remarkable, our next plan is to perform the animal study (*in vivo*) using the rats to address particularly the safety, chemosensitizing effect, and overall efficacy of LLE with drugs (CPL etc.).

5. Conclusion

The present study shows that anticancer activity of CPL can be significantly enhanced with LLE. This (selective) chemosensitizing effect is more likely attributed to the glycolysis inhibition, a G_1 cell cycle arrest, and chromatin modifications, ultimately leading to apoptosis. Therefore, certain *natural products* such as LLE could be used as an adjuvant agent in current chemotherapy, improving the drug efficacy but minimizing side effects. Further studies are warranted.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Zhang, Y., Rumgay, H., Li, M., Yu, H., Pan, H. and Ni, J. (2023) The Global Landscape of Bladder Cancer Incidence and Mortality in 2020 and Projections to 2040. *Journal of Global Health*, **13**, Article 04109. https://doi.org/10.7189/jogh.13.04109

[2] Siegel, R.L., Giaquinto, A.N. and Jemal, A. (2024) Cancer Statistics, 2024. *CA*: *A Cancer Journal for Clinicians*, **74**, 12-49. https://doi.org/10.3322/caac.21820

[3] Kaufman, D.S., Shipley, W.U. and Feldman, A.S. (2009) Bladder Cancer. *The Lancet*, **374**, 239-249. https://doi.org/10.1016/s0140-6736(09)60491-8

[4] Shen, Z., Shen, T., Wientjes, M.G., O'Donnell, M.A. and Au, J.L.-S. (2008) Intravesical Treatments of Bladder Cancer: Review. *Pharmaceutical Research*, **25**, 1500-1510. https://doi.org/10.1007/s11095-008-9566-7

[5] Malmström, P. (2003) Intravesical Therapy of Superficial Bladder Cancer. *Critical Reviews in Oncology/Hematology*, **47**, 109-126. https://doi.org/10.1016/s1040-8428(03)00075-1

[6] Bukowski, K., Kciuk, M. and Kontek, R. (2020) Mechanisms of Multidrug Resistance in Cancer Chemotherapy. *International Journal of Molecular Sciences*, **21**, Article 3233. https://doi.org/10.3390/ijms21093233

[7] Racioppi, M., D'Agostino, D., Totaro, A., Pinto, F., Sacco, E., D'Addessi, A., *et al*. (2012) Value of Current Chemotherapy and Surgery in Advanced and Metastatic Bladder Cancer. *Urologia Internationalis*, **88**, 249-258. https://doi.org/10.1159/000335556

[8] Suganuma, M., Saha, A. and Fujiki, H. (2010) New Cancer Treatment Strategy Using Combination of Green Tea Catechins and Anticancer Drugs. *Cancer Science*, **102**, 317-323. https://doi.org/10.1111/j.1349-7006.2010.01805.x

[9] Dasari, S., Njiki, S., Mbemi, A., Yedjou, C.G. and Tchounwou, P.B. (2022) Pharmacological Effects of Cisplatin Combination with Natural Products in Cancer Chemotherapy. *International Journal of Molecular Sciences*, **23**, Article 1532. https://doi.org/10.3390/ijms23031532

[10] Cicero, A.F.G., Allkanjari, O., Busetto, G.M., Cai, T., Larganà, G., Magri, V., *et al*. (2019) Nutraceutical Treatment and Prevention of Benign Prostatic Hyperplasia and Prostate Cancer. *Archivio Italiano di Urologia e Andrologia*, **91**, 139-152. https://doi.org/10.4081/aiua.2019.3.139

[11] Li, C., Lin, L., Sui, F., Wang, Z., Huo, H., Dai, L., *et al*. (2014) Chemistry and Pharmacology of Siraitia Grosvenorii: A Review. *Chinese Journal of Natural Medicines*, **12**, 89-102. https://doi.org/10.1016/s1875-5364(14)60015-7

[12] Haung, R., Saji, A., Choudhury, M. and Konno, S. (2023) Potential Anticancer Effect of Bioactive Extract of Monk Fruit (*Siraitia grosvenori*) on Human Prostate and Bladder Cancer Cells. *Journal of Cancer Therapy*, **14**, 211-224. https://doi.org/10.4236/jct.2023.145019

[13] Chiu, C., Wang, R., Lee, C., Lo, Y. and Lu, T. (2013) Biotransformation of Mogrosides from *Siraitia grosvenorii* Swingle by *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry*, **61**, 7127-7134. https://doi.org/10.1021/jf402058p

[14] Liu, C., Dai, L., Liu, Y., Rong, L., Dou, D., Sun, Y., *et al*. (2016) Antiproliferative Activity of Triterpene Glycoside Nutrient from Monk Fruit in Colorectal Cancer and Throat Cancer. *Nutrients*, **8**, Article 360. https://doi.org/10.3390/nu8060360

[15] Liu, C., Dai, L., Liu, Y., Dou, D., Sun, Y. and Ma, L. (2018) Pharmacological Activities of Mogrosides. *Future Medicinal Chemistry*, **10**, 845-850. https://doi.org/10.4155/fmc-2017-0255

[16] Takasaki, M., Konoshima, T., Murata, Y., Sugiura, M., Nishino, H., Tokuda, H., *et al*. (2003) Anticarcinogenic Activity of Natural Sweeteners, Cucurbitane Glycosides, from *Momordica grosvenori*. *Cancer Letters*, **198**, 37-42. https://doi.org/10.1016/s0304-3835(03)00285-4

[17] Xu, Q., Chen, S.Y., Deng, L.D., Feng, L.P., Huang, L.Z. and Yu, R.R. (2013) Antioxidant Effect of Mogrosides against Oxidative Stress Induced by Palmitic Acid in Mouse Insulinoma NIT-1 Cells. *Brazilian Journal of Medical and Biological Research*, **46**, 949-955. https://doi.org/10.1590/1414-431x20133163

[18] Suzuki, Y.A., Tomoda, M., Murata, Y., Inui, H., Sugiura, M. and Nakano, Y. (2007) Antidiabetic Effect of Long-Term Supplementation with *Siraitia grosvenori* on the Spontaneously Diabetic Goto-Kakizaki Rat. *British Journal of Nutrition*, **97**, 770-775. https://doi.org/10.1017/s0007114507381300

[19] Pelicano, H., Martin, D.S., Xu, R. and Huang, P. (2006) Glycolysis Inhibition for Anticancer Treatment. *Oncogene*, **25**, 4633-4646. https://doi.org/10.1038/sj.onc.1209597

[20] Spitz, D., Simons, A., Mattson, D. and Dornfeld, K. (2009) Glucose Deprivation-Induced Metabolic Oxidative Stress and Cancer Therapy. *Journal of Cancer Research and Therapeutics*, **5**, S2-S6. https://doi.org/10.4103/0973-1482.55133

[21] Miccoli, L., Oudard, S., Sureau, F., Poirson, F., Dutrillaux, B. and Poupon, M. (1996) Intracellular pH Governs the Subcellular Distribution of Hexokinase in a Glioma Cell Line. *Biochemical Journal*, **313**, 957-962. https://doi.org/10.1042/bj3130957

[22] Loar, P., Wahl, H., Kshirsagar, M., Gossner, G., Griffith, K. and Liu, J.R. (2010) Inhibition of Glycolysis Enhances Cisplatin-Induced Apoptosis in Ovarian Cancer Cells. *American Journal of Obstetrics and Gynecology*, **202**, 371.E1-E8. https://doi.org/10.1016/j.ajog.2009.10.883

[23] Sherr, C.J. (2000) The Pezcoller Lecture: Cancer Cell Cycles Revised. *Cancer Research*, **60**, 3689-3695.

[24] Goranov, A.I., Cook, M., Ricicova, M., Ben-Ari, G., Gonzalez, C., Hansen, C., *et al*. (2009) The Rate of Cell Growth Is Governed by Cell Cycle Stage. *Genes & Development*, **23**, 1408-1422. https://doi.org/10.1101/gad.1777309

[25] Gao, Y. and Tollefsbol, T. (2018) Combinational Proanthocyanidins and Resveratrol Synergistically Inhibit Human Breast Cancer Cells and Impact Epigenetic-Mediating Machinery. *International Journal of Molecular Sciences*, **19**, Article 2204. https://doi.org/10.3390/ijms19082204

[26] Yip, K.W. and Reed, J.C. (2008) Bcl-2 Family Proteins and Cancer. *Oncogene*, **27**, 6398-6406. https://doi.org/10.1038/onc.2008.307

[27] Paine, M.F. (2020) Natural Products: Experimental Approaches to Elucidate Disposition Mechanisms and Predict Pharmacokinetic Drug Interactions. *Drug Metabolism and Disposition*, **48**, 956-962. https://doi.org/10.1124/dmd.120.000182

[28] Dennis, T., Fanous, M. and Mousa, S. (2009) Natural Products for Chemopreventive and Adjunctive Therapy in Oncologic Disease. *Nutrition and Cancer*, **61**, 587-597. https://doi.org/10.1080/01635580902825530