RESEARCH ARTICLE

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Hesperidin and Diosmin Increased Cytotoxic Activity Cisplatin on Hepatocellular Carcinoma and Protect Kidney Cells Senescence

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Abstract

Objective: Cisplatin (Cisp) is a chemotherapy drug for treating liver cancer. Hesperidin (HSD), a flavanone, is known for its anticancer, and anti-inflammatory properties. Diosmin (DSM), a flavone glycoside, is known for its anti-oxidant effect. This research investigated the synergism cytotoxic effect and senescence selectivity effect of HSD or DSM co-treatment with Cisp on HepG2 cells and Vero cells. **Methods:** The cytotoxicity and cell viability of HSD or DSM combined with Cisp on HepG2 and Vero cells were assessed using the MTT assay with IC_{50} parameters, selectivity index (SI), and Combination Index (CI), while the antiproliferative profile was determined by colony-forming assay. Cellular senescence on HepG2 and Vero cell lines was determined using senescence-associated β-galactosidase (SA-β-gal) staining. Furthermore, the impact of apoptosis was evaluated using flowcitometry. **Result:** In the MTT assay, HSD, DSM, and cisplatin exhibited cytotoxic effects on HepG2 cells, with IC_{50} values of 321 μM, 148 μM, and 5 μM, respectively. Co-treatment with HSD and DSM with cisplatin enhanced cell sensitivity, resulting in a combination index of < 1. HSD and DSM exhibited minimal cytotoxicity against Vero cells, with IC_{50} values exceeding 500 μM. Both HSD and DSM reduced cellular senescence in Vero cells caused by cisplatin exposure. These findings suggest that co-treatment with HSD and DSM alongside cisplatin can synergistically lessen the viability of HepG2 cells. The Annexin V-FITC/PI apoptosis assay also showed more cells undergoing apoptosis in the co-treatment group. Both co-treatment HSD and DSM with Cisp independently induced the senescence of HepG2 cells and reduced the cellular senescence of normal kidney cells. **Conclusion:** Consequently, both HSD and DSM show potential for development as co-treatment agents in combination with Cisp for hepatocellular carcinoma, and they may also be useful for reducing senescence in normal kidney cells.

Keywords: hesperidin- diosmin- cisplatin- HepG2- Vero

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Introduction

Hepatocellular carcinoma (HCC) is a primary liver tumor, accounting for over 90% of such tumors. It develops in about 85% of patients with cirrhosis. Globally, HCC is now the fifth most common cancer [1]. Cisplatin (Cisp), a well-known classical chemotherapeutic drug, is commonly used to treat HCC [2]. Approved as an anticancer agent in the 1970s, it was the first platinum-based drug and, like other platinum compounds, demonstrates a broad spectrum of activity. These drugs are highly effective in treating various solid tumors, including testicular, ovarian, head and neck, bladder, lung, cervical cancers, melanoma, and lymphoma. They usually used to treat over 80% of cancers [3]. Although numerous drugs have been approved for treating HCC, the development of resistance to Cisp has led to inadequate drug responses in HCC patients [4]. Additionally, it causes numerous adverse effects, including cytotoxicity, fever, abdominal pain, infection, nausea, weakness, and fatigue. The kidneys and auditory organs with nephrotoxicity are common and have severe side effects [5]. Consequently, extensive research has been conducted to discover a safer kidney and more effective alternative anticancer agent for treating HCC.

Natural substances and their active compounds are crucial in managing diseases and tend to have fewer side effects [6]. Recent studies on the anticancer effects of natural products have concentrated on inducing cancer cell death. This cell death, whether triggered by chemotherapy or natural products, can occur through apoptosis [7].

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Previous research has shown that natural compounds or their key components can enhance health by lowering oxidative stress and inflammation and helping regulate cell senescence pathways [8]. Hesperidin (HSD) is the most abundant flavanone in Citrus peel, a category belonging to the commonly found and extensively spread plant phenolic compounds. Its importance in health management has been acknowledged due to its antioxidant, anti-inflammatory, and anti-cancer properties and its ability to prevent senescence [9]. Diosmin (DSM) is the glycosylated flavone in small quantities in Citrus peel [10]. Containing a highly active flavonoid, it has been shown to possess anti-inflammatory, free-radical scavenging, and antimutagenic properties [11]. DSM has been studied in various animal models and human cancer cell lines, showing potential chemopreventive and antiproliferative effects also reported to promote apoptosis [12]. The inhibition of apoptosis is a critical feature in cancer progression, underscoring the importance of inducing apoptosis in cancer cells as an effective treatment approach. Genetic control over apoptosis impacts cell survival, and mutations disrupting this process can contribute to the formation of cancers [13].

Certain chemotherapy treatments are combined with other drugs. HSD and DSM have been demonstrated to enhance the efficacy of chemotherapy, including treatments involving doxorubicin and cisplatin, against different cancer cells However, no studies have been conducted on co-treatment with Cisp in HCC and its effect on senescence in normal kidney cells. Combination therapies using multiple drugs have gained popularity in cancer treatment. According to these studies, HSD and DSM appear to improve the response of HepG2 cells to cisplatin. This research aimed to evaluate the combinative effect of cisplatin and HSD or DSM on HCC cell lines and also a senescence effect on normal kidney cells.

Materials and Methods

Cell culture

HepG2 hepatocellular carcinoma (ATCC® HB-8065) and Vero normal kidney (ATCC® CCL-81) cells were obtained from the Cancer Chemoprevention Research Center in Yogyakarta, Indonesia. These cells were cultured using Dulbecco's Modified Eagle Medium (DMEM) medium enriched with 10% fetal bovine serum (Sigma, St. Louis, USA), 1% penicillin/streptomycin (Gibco), and 0.5% Fungizone (Corning), in a humidity-controlled incubator that maintained an atmosphere of 95% air and 5% carbon dioxide at 37°C. The growth medium was refreshed every three to four days, with cells being transferred or passaged once they reached 80–90% confluence. Trypsin-EDTA was employed for cell subculturing to assist in their release. The compounds used in the research included Hesperidin (Sigma), Diosmin (Sigma), and Cisplatin (Wako).

Cell Proliferation MTT Assay

The MTT colorimetric assay, utilizing the microculture tetrazolium technique with MTT (from Sigma, St. Louis, USA) as outlined by Mosmann, was applied to determine the cytotoxic effects of HSD, DSM, and Cisp in vitro. In summary, cells at a density of 1×10^4 per well were treated with different HSD, DSM, or Cisp concentrations and incubated for 24 hours. An MTT reagent solution (0.5 mg/mL from Biovision) was then administered at 100 µL per well, and the cells were incubated again for 2 to 4 hours. Subsequently, a solution containing Sodium Dodecyl Sulfate (SDS) and 0.01N HCl was added to halt the reaction. Absorbance at 595 nm was measured via an ELISA plate reader. Cell survivability was deduced from these absorbance values. The half-maximal inhibitory concentration, or IC_{50}, was ascertained through linear regression to associate the substance concentration with the cells' survival percentage. The selectivity index (SI), indicates the cytotoxic selectivity. The formula for calculating SI is dividing the IC_{50} of the pure compound in a normal cell line by the IC_{50} of the same pure compound in a cancer cell line [14]. A high SI value (>2) of a compound gives selective toxicity toward cancer cells [15]. Effects of combinations on growth inhibition were analyzed using the combination index (CI) equation developed by Reynolds and Maurer, 2005 [16].

Colony Formation Assay

Cells were plated at a concentration of 500 cells in each well of a 24-well plate and allowed to grow for 24 hours. Following this initial incubation, the cells underwent a 24-hour drug treatment. Subsequently, the old medium was exchanged for new, and the cells were incubated for another eight days. After this period, cells were secured in place with paraformaldehyde and colored with a Gentian violet (0.5% w/v) stain. The resultant cell colonies were then tallied and examined using the ColonyArea software.

Senescence-associated β-galactosidase (SA-βGal) Senescence-based Assay

Evaluation of β-galactosidase activity, which signifies cellular aging, was done using the SA-βGal technique. For this process, Vero and HepG2 cells were cultured at 2×10^4 cells per well in a 24-well plate and left to grow for a day. The cells were then cleansed two times with PBS at 1× concentration. They were treated with a fixing solution for a set duration and rewashed with 1× PBS. Next, the cells were covered with 650 µL of an X-Gal staining solution and maintained at 37°C. Following the 72 hours, the cells were inspected using an Olympus CKX-41 microscope at a magnification of 200 times. The appearance of blue-stained cells, indicative of senescence, identified β-galactosidase activity.

Apoptosis Detection

The presence of apoptotic cell populations was assessed using a PI-Annexin V assay, employing the Annexin V-FITC Apoptosis Detection Kit from Roche Mannheim, Germany. HepG2 cells were plated at a density of 2×10^5 cells per well in 6-well plates and exposed to varying levels of the test substances alone or in combination. Following 24 hours of treatment, the cells were collected, mixed with binding buffer at a 1x concentration, tagged with PI-Annexin V, and left to incubate at room temperature away from light for

5 minutes. Subsequently, the suspension of cells was subjected to flow cytometry analysis utilizing equipment from BD Biosciences.

Statistical analysis

The data were shown as mean ± SD and analyzed with SPSS 21.0 software. One-way ANOVA was used to assess the statistical significance of differences between the untreated and various treatment groups. The corresponding p-values (*p<0.05; **p<0.01 ***p<0.001) are marked in each experimental figure.

Results

Effect of HSD, DSM, and Cisplatin on Cell Viability of HepG2 cells and Vero cell

The study aims to investigate HSD, DSM, and cisplatin (Cisp) regarding various cancer hallmarks and their selectivity for normal cells in vitro. Initially, we assessed the cytotoxic effects of HSD, DSM, and Cisp on HepG2 hepatoblastoma cells and Vero cells over 24 hours. HepG2 cells served as a model for HCC, while Vero cells were used for normal kidney cells. HSD and DSM, at concentrations ranging from 20-400 µM, reduced the viability of HepG2 cells in a dose-dependent manner, with IC_{50} values of 321 µM and 148 µM, respectively, suggesting they are potentially weak cytotoxic agents [17]. HSD showed no cytotoxic effect on Vero cells at concentrations up to 1,000 µM, and DSM showed no cytotoxic effect on Vero cells at concentrations up to 500 µM. These findings indicate that HSD and DSM exhibit selective cytotoxicity towards cancer cells, with a selectivity index greater than 2 (Figure 1).

Cytotoxic Effect of Combination of HSD or DSM with Cisplatin on HepG2 Cells

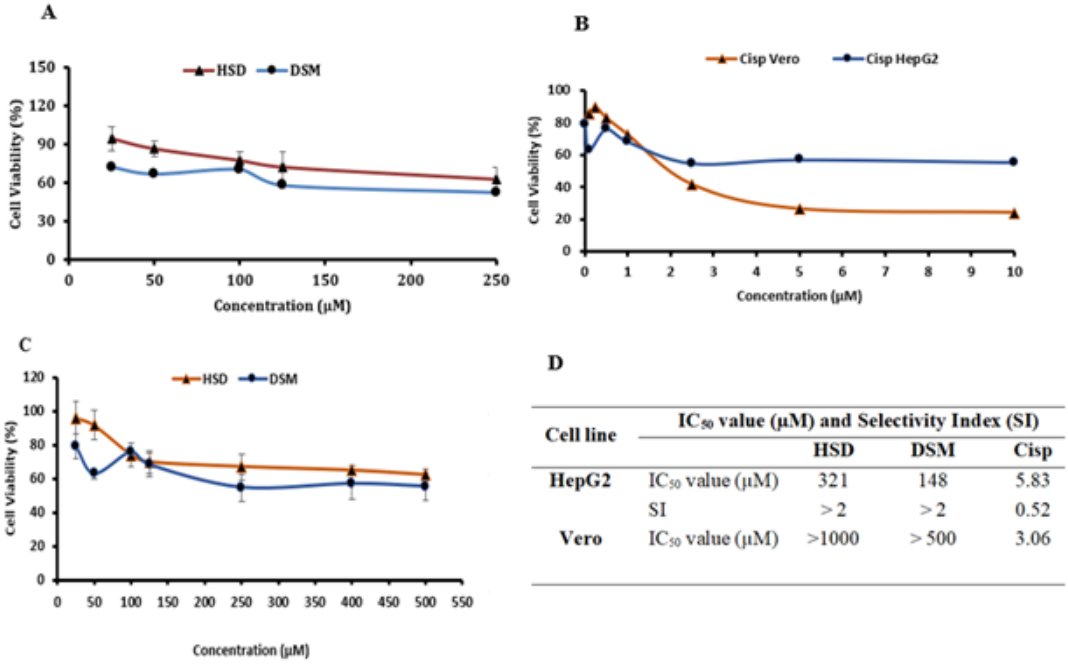
In a single cytotoxicity test, HSD and DSM demonstrated strong potential despite their weak cytotoxicity. Subsequently, we investigated the cytotoxicity of the combination with cisplatin (Cisp) to uncover its synergistic effects against HCC (Figures 2A and 2B). The results indicated that HSD, DSM, and Cisp exhibited good synergistic properties on HepG2 cells in inhibiting cell growth with a CI value of less than 1 (Figure 2C).

HSD, DSM Combination with Cisplatin Suppressed HepG2 Cell Survival Viability

Hepatocytes cultured in vitro can be sustained as replicating differentiated cells for a restricted duration. The colony-forming or clonogenic assay is an in vitro quantitative technique used to evaluate the capacity of a single cell to proliferate and form a large colony through clonal expansion. It is the preferred method for assessing the efficacy of other cytotoxic agents [18]. The clonogenic assay revealed that HSD and co-treatment with cisplatin (Cisp) (Figures 3A and 3C), and DSM co-treatment with Cisp suppressed cell survival viability from HepG2 cells compared with the untreated group after treatment 8 days (Figures 3B and 3D).

Senescence Effect of Combination of HSD, DSM with Cisplatin on HepG2 Cells

The recent discovery that various anticancer agents also induce cellular senescence suggests that pro-senescence strategies could be useful in cancer prevention therapy.



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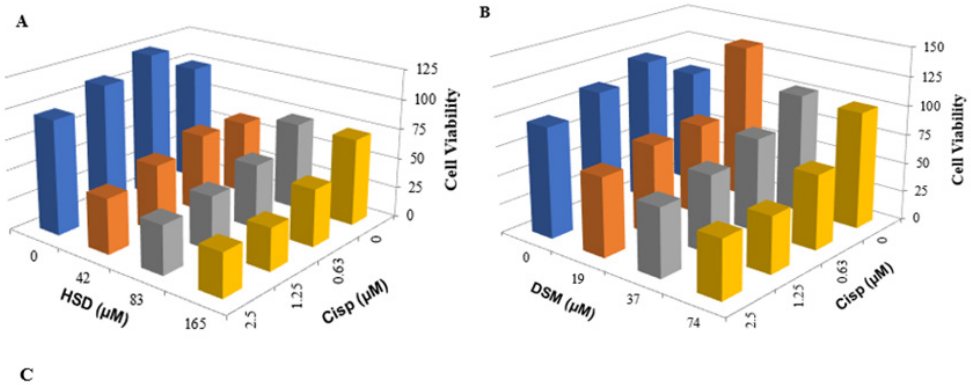
Cell line	IC_{50} value (µM) and Selectivity Index (SI)			
		HSD	DSM	Cisp
HepG2	IC_{50} value (µM)	321	148	5.83
	SI	>2	>2	0.52
Vero	IC_{50} value (µM)	>1000	>500	3.06

Figure 1. Effect of HSD, DSM, and Cisp on the HepG2 Cell Viability. A. Effect of HSD and DSM on the viability of HepG2 cells. Cells were cultured and treated with increasing concentrations of HSD (25-250 µM) and DSM (25-250µM) B. Effect of Cisp on the viability of Vero and HepG2 cells. Cells were cultured and treated with cisplatin (Cisp) (0.01-10 µM) for 24 hours in DMEM containing 10% FBS. C. Effect of HSD and DSM at concentration of 25-500 µM on the viability of Vero cells. Cell viability was assessed by MTT assay in triplicate (n=3). D. The IC_{50} value and Selectivity Index (SI) of HSD, DSM, and Cisp. Data represent the means ± SEM



C

Combination Index in HepG2 cells			
	HSD (µM)		
Cisp (µM)	42	83	165
0.6	0.38	0.24	0.21
1.2	0.29	0.06	0.02
2.5	0.41	0.06	0.01

Combination Index in HepG2 cells			
	DSM (µM)		
Cisp (µM)	19	37	74
0.6	0.56	0.77	0.47
1.2	0.87	0.54	0.42
2.5	0.89	0.68	0.65

Figure 2. The Combined Effect of the HSD or DSM Co-Treatment with Cisp on HepG2 Cells. A. Combination effect of HSD and Cisp on the viability of HepG2 cells. B. Combination effect of DSM and Cisp on the viability of HepG2 cells. C. The combination Index (CI) value of HSD or DSM with Cisp.

In this context, we investigated the effect of co-treatment HepG2 cells with HSD, DSM, and cisplatin (Cisp) on the induction of cellular senescence. The observations were also carried out at 24 hours. The results confirmed that HSD co-treatment with Cisp at concentrations of 83 and 165 µM significantly increased the incidence of cellular senescence in HepG2 cells (Figures 4A and 4C). Co-treatment of DSM with Cisp at concentrations of 37 and 74 µM notably enhanced the occurrence of cellular senescence in HepG2 cells (Figures 4B and 4D). These

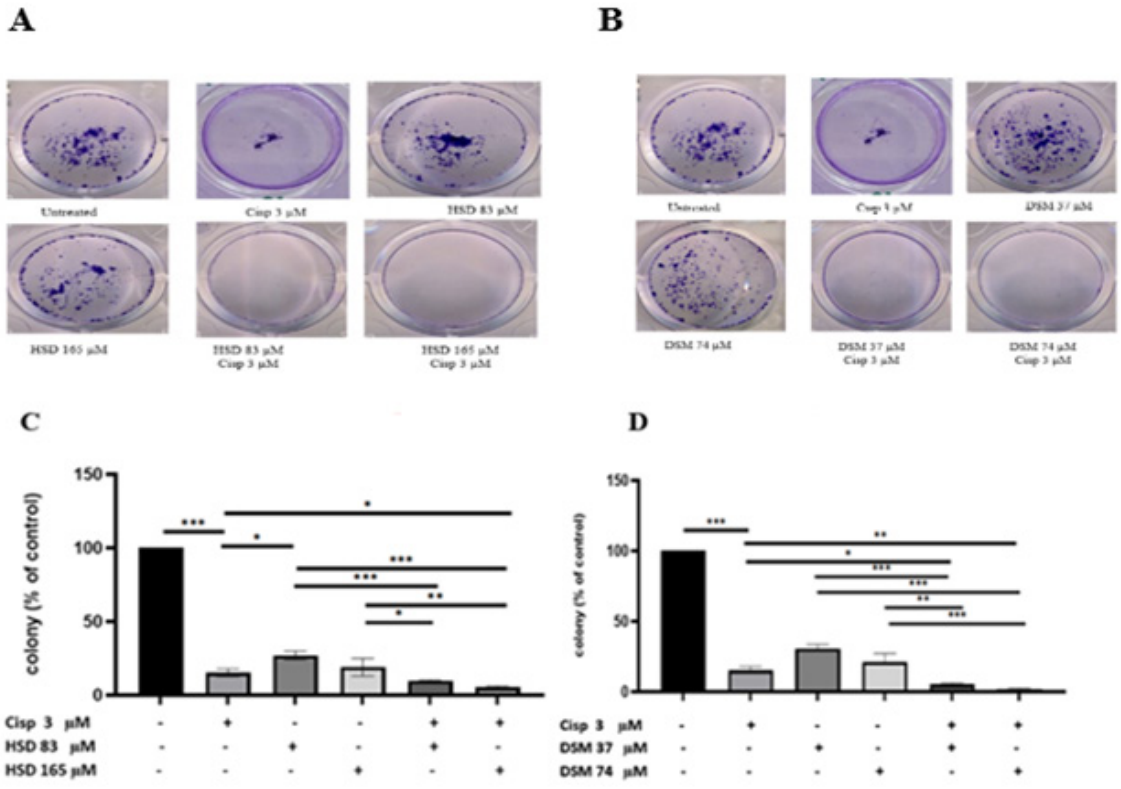


Figure 3. Effects of Combined Treatment of HSD, DSM, and Cisp in the ColonyFormation of HepG2 Cells. The colony formation assay was performed as described in the methods section, cells were treated with HSD (A), DSM (B), or Cisp and the combination as indicated in the graph. The surviving colonies were counted and analyzed using ImageJ software. (A) HSD (B) DSM and the quantification of (C) HSD (D) DSM on HepG2 cells were displayed as mean±SD (n=3). ***: p ≤0.001 ** : p ≤0.01 * : p ≤0.1.

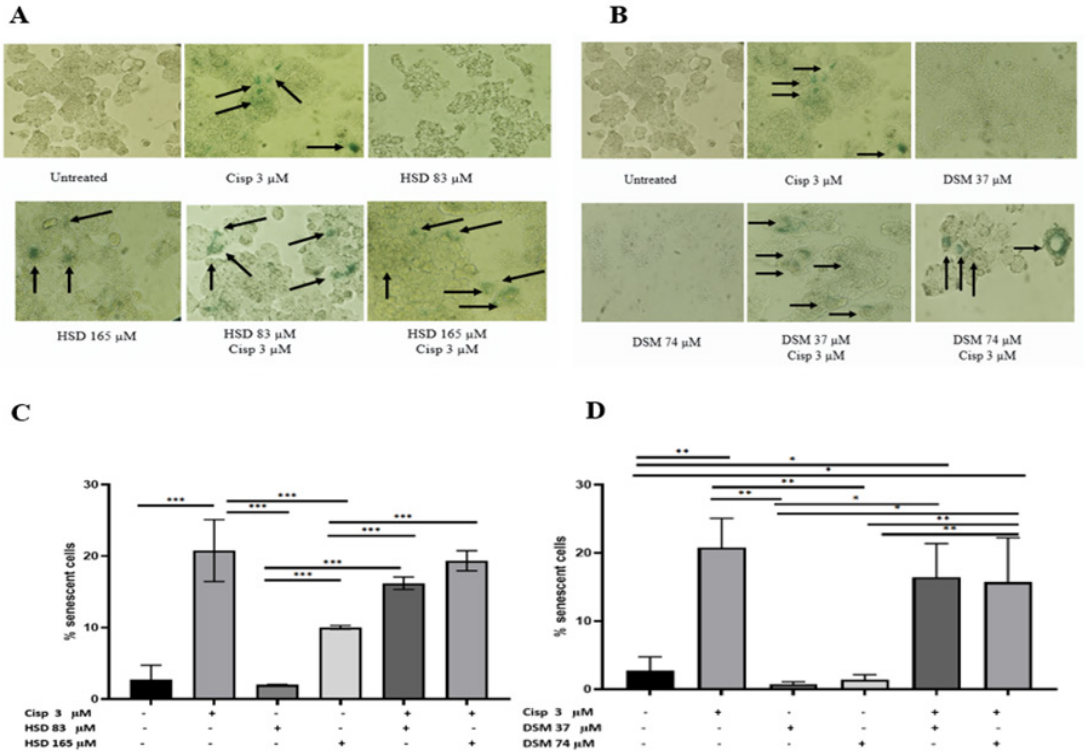


Figure 4. The Senescence Effect of the HSD or DSM Co-Treatment with Cisp on HepG2 Cells. Cells were seeded and treated with samples, (A) HSD, (B) DSM, or its combination with Cisp, then proceeded for senescence assay. The arrows show β-galactosidase-positive cells, which indicate the senescent cells. Quantification of senescent cells under treatment, (C) HSD (D) DSM and its combination with Cisp on HepG2 cells. ***: p ≤0.001 ** : p ≤0.01 * : p ≤0.1.

results corroborate prior studies highlighting the impact of combined HSD and DSM treatment with Cisp in promoting cellular senescence in cancer cells and its cytotoxic effects.

Apoptosis Induction of the Combination of HSD, DSM with Cisplatin on HepG2 Cells

The effect of HSD and DSM co-treatment with cisplatin (Cisp) on cell apoptosis was analyzed using flow cytometry following sample preparation and staining with Annexin V and PI reagents. This method made distinguishing between cells undergoing early apoptosis, late apoptosis, and necrosis possible. Treatment with 3 µM Cisp increased cell apoptosis by 2.51% compared to normal cells. A single treatment with 100 µM HSD led to a 1.18% increase in cell apoptosis, while the combination treatment resulted in a 5.28% increase. A single treatment with 100 µM DSM led to a 2.31% increase in cell apoptosis, while the combination treatment resulted in a 2.54% increase (Figure 5B). The combination treatment increased cell apoptosis compared to the single treatment with Cisp and the untreated cells. However, the

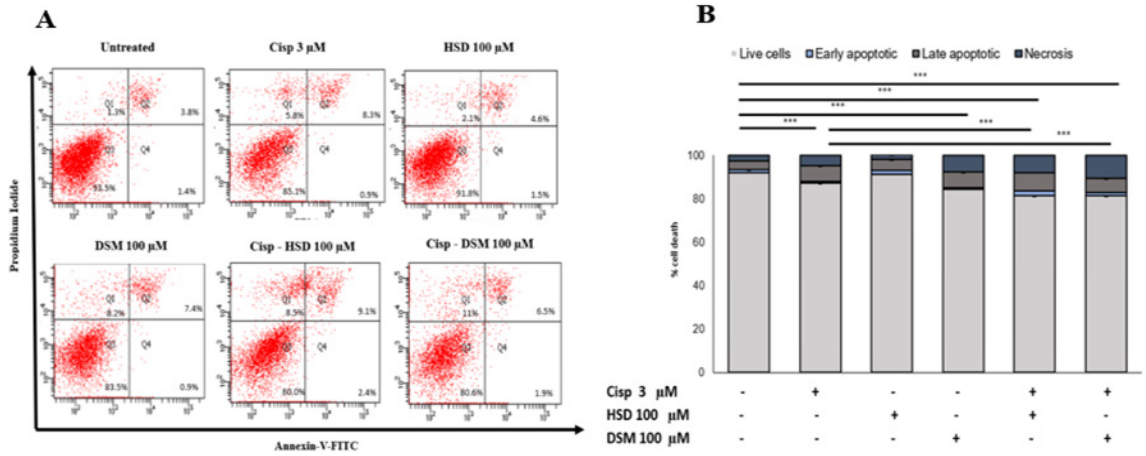
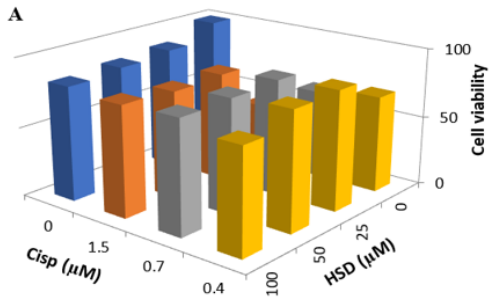
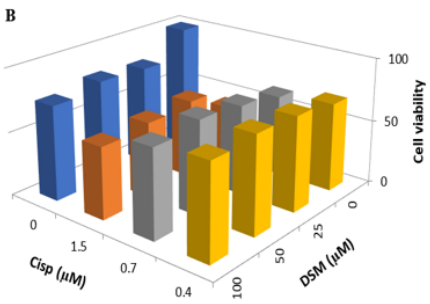


Figure 5. Induction of Apoptosis by HSD, DSM, and Its Combination with Cisp on HepG2 Cells. Cells were exposed to HSD, DSM, Cisp, or their combination as indicated in the graph for 24 h. Percentage cell death (A, B) was measured by flow cytometry after staining with Annexin V-FITC and PI, then classified into early apoptosis, late apoptosis, and necrosis. The bar represents the mean ± SD value from the triplicate independent experiment. ***: p < 0.001.





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Combination Index in Vero cells			
	HSD (μM)		
Cisp (μM)	25	50	100
0.4	45.6	43.4	23.2
0.7	85.9	12.0	25.5
1.5	32.2	57.1	14.3

Combination Index in Vero cells			
	DSM (μM)		
Cisp (μM)	25	50	100
0.4	7.43	19.63	21.64
0.7	8.30	18.98	25.57
1.5	12.02	21.62	38.99

Figure 6. The Cytotoxic Effect of HSD or DSM Combination with Cisp on Vero Cells. A. Combination effect of HSD and Cisp on the viability of Vero cells. B. Combination effect of DSM and Cisp on the viability of Vero cells C. The combination Index (CI) value of HSD or DSM combination with Cisp.

number of living cells remained high, exceeding 80%. According to the cytotoxicity assay, the HSD, DSM, and Cisp combination induced apoptosis. Further research is required to investigate the ability of HSD and DSM to induce senescence in HepG2 cells.

Effect of Combination of HSD, DSM, and Cisplatin on Cell Viability of Vero Cells

Additionally, we tested the co-treatment of HSD and DSM with cisplatin (Cisp) in Vero normal kidney cells despite HSD and DSM being weakly cytotoxic. Contrary to their cytotoxic effect on HepG2 cells, HSD and DSM exhibited an antagonistic effect when combined with Cisp in Vero cells (Figures 6A and 6B). It inhibits cell growth with a CI value of more than 1 (Figure 6C).

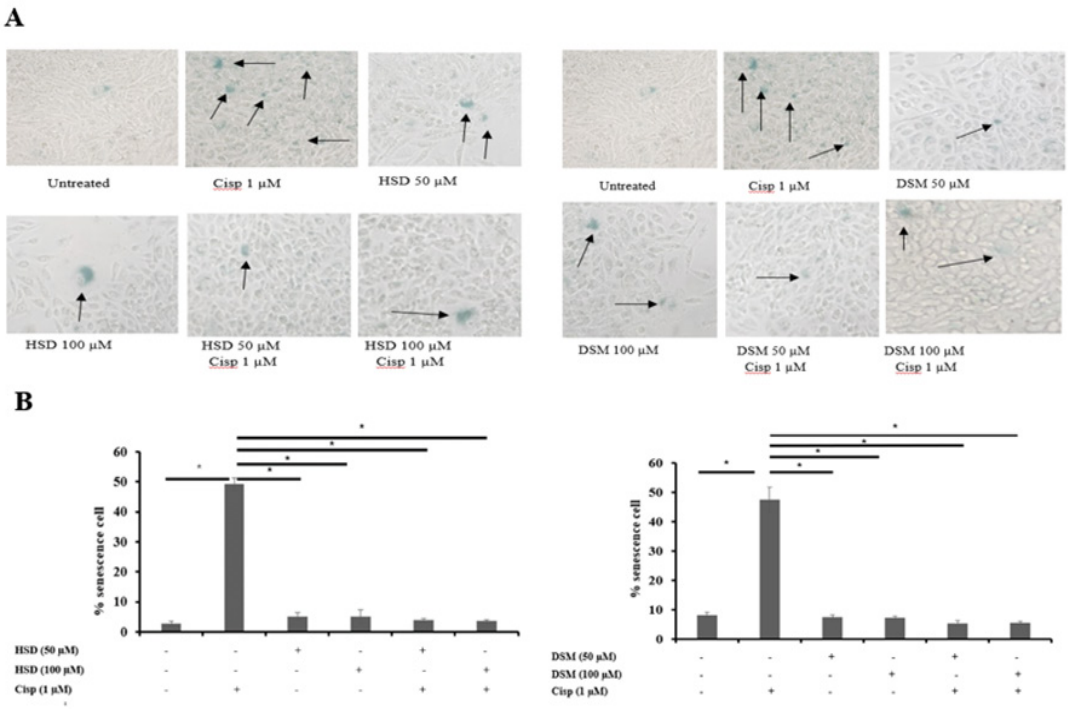

Figure 7. The Anti-Senesence Effect of the HSD (A) or DSM (B) Co-Treatment with Cisp on Vero Cells. Cells were seeded and treated with samples, HSD, DSM, or its combination with Cisp, as indicated in the figure, then proceeded for senescence assay. (A) Cells that experience senescence under treatment. The arrows show β-galactosidase-positive cells, which are expressed in senescent cells. (B) Quantification of Vero cells undergoing senescence. ***: $p \leq 0.001$ **: $p \leq 0.01$ *: $p \leq 0.1$.

Senescence Effect of Combination of HSD, DSM with Cisplatin on Vero cells

Cisplatin (Cisp) administration causes side effects that lead to cellular damage, resulting in abnormalities and potential harm to the kidneys. Prolonged exposure to substances that harm the kidneys can ultimately lead to chronic kidney failure [19]. We examined its effects on inducing senescence in normal kidney cells, specifically using Vero cells. Cisplatin (Cisp) significantly increased the number of β-galactosidase-positive cells, indicating that Cisp induces senescence in Vero cells ($p < 0.01$). In contrast, co-treatment with HSD and DSM alongside Cisp did not result in any changes in β-galactosidase-positive cells compared to the untreated cells (Figure 7A and 7B). These findings indicate that HSD and DSM protect normal kidney cells from the senescence induced by Cisp.

Discussion

Nephrotoxicity caused by chemotherapy has led to the development of new compounds used in combination with chemotherapeutic agents, known as co-chemotherapy. Fruits and vegetables rich in flavonoids are frequently suggested to lower cancer risk. Some of these flavonoids are also being explored as potential treatments for cancer [13]. Multiple studies have shown that HSD, a citrus flavanone, exhibits various pharmacological activities, including antioxidant properties, cytotoxicity against several cancer cell lines, induction of apoptosis, and anti-inflammation [20]. Similarly, DSM, a citrus polyhydroxyflavone, has been reported to reduce inflammation, prevent oxidative stress, and induce apoptosis in colon cancer [21]. Considering the many promising qualities of flavonoids, this study hypothesized that their therapeutic potential would be significantly enhanced when combined. A compound combined with chemotherapy should be effective and less toxic to normal cells [22]. HSD and DSM possess favorable pharmacodynamic and pharmacokinetic properties in the human body and do not exhibit significant toxic effects in animals [23]. Therefore, these findings support using HSD and DSM as combination agents in HCC therapy.

This study investigated HSD and DSM as anticancer agents against HepG2 cells as HCC to assess their cytotoxicity and potential to protect senescence when combined with Cisp. Based on these findings, the co-treatment of HSD or DSM with Cisp exhibited a stronger inhibitory effect than HSD and DSM alone on HCC. In the equimolar drug combination, IC_{50} values of 42, 83, and 165 μM for HSD with 0.63, 1.25, and 2.5 μM for Cisp, as well as 19, 37, and 74 μM for DSM with 0.63, 1.25, and 2.5 μM for Cisp, were observed in HepG2 cells. Additionally, CI analysis was conducted to evaluate the synergistic effect of the co-treatment of HSD or DSM with Cisp on HepG2 cells. The combined treatment exhibited a broad range of synergism, with CI values ranging from 0.01 to 0.41 of HSD and 0.42-0.89 of DSM in HepG2 cells. This indicates that co-treatment of HSD or DSM with Cisp had a beneficial impact on cytotoxicity in HCC. HSD interacts with several known cellular targets and suppresses the growth of malignant stem cells by inducing apoptosis and halting the cell cycle [24]. DSM suppressed the proliferation of MDA-MB-231 cells in a concentration- and time-dependent manner, showing no toxicity toward normal human epithelial cells in vitro. It promoted apoptosis by raising the Bax/Bcl-2 ratio and enhancing active caspase-3 levels [25]. HSD offers a potential synergistic effect that improves the anticancer effectiveness of doxorubicin while minimizing the risks associated with chemotherapy in highly metastatic breast cancer [26]. DSM enhances the anti-migration activity of PGV-1 in WiDr cells, potentially by modulating MMP activity [27]. Therefore, this study showed that the combined HSD and DSM with Cisp treatment inhibited cell proliferation synergistically on HCC.

Many factors evaluated in antiproliferative assays, such as drug uptake, activity in the presence of competing cellular binding sites, and stability, are also pertinent to clonogenic assays. Similarly, interactions with sites other than the target for cytotoxicity may be so significant that cell-based assays become essential [28]. Clonogenic assays encompass various cell death pathways and are especially valuable for measuring cell survival after exposure to cytotoxic drugs [29]. The clonogenic assay revealed that HSD or DSM co-treatment with Cisp suppressed cell survival viability from HepG2 cells compared with the untreated group after treatment 8 days. The cells are stopped from surviving and multiplying, causing them to enter a state of senescence. Cellular senescence is when cells cease to divide but continue to be metabolically active, entering a state of irreversible cell cycle arrest [30]. Cisplatin was used to induce cellular senescence [31]. In this study, we treated HepG2 cells with 3 μM of Cisp, combined with either 83 μM or 165 μM of HSD, and with either 37 μM or 74 μM of DSM. The results indicated that combining cisplatin with HSD or DSM exhibited a more pronounced senescent phenotype on HepG2 cells than a single HSD or DSM treatment.

Senescence and apoptosis are two cellular responses to damage modified in cancerous and normal cells, albeit through different mechanisms [32]. When cell viability is suppressed, it often means the cell's ability to survive and proliferate is compromised. This can lead to the activation of apoptosis as a natural mechanism to remove damaged cells from the body. Apoptosis is a crucial physiological process for tissue development and homeostasis [33]. One of the defining characteristics of cancer is the suppression of apoptosis [34]. Therefore, the prevailing model suggests that apoptosis and cellular senescence are critical tumor-suppressor mechanisms [35]. Induction of cell apoptosis is typically most prominent at the highest drug doses.

In contrast, lower doses of chemotherapeutic drugs tend to have a more pronounced cytostatic effect, causing senescence with temporary growth arrest. The current study also demonstrated the induction of nuclear apoptosis using fluorochrome-labeled Annexin V/PI through flow cytometry. During apoptosis, phosphatidylserine (PS), typically found on the inner membrane of living cells, translocates to the outer membrane. Annexin V binds strongly to PS, allowing flow cytometry to quantify

viable and apoptotic cells [36]. In this study, untreated cells showed the highest number of viable cells, whereas treatments with HSD, DSM, and co-treatment of HSD and DSM with Cisp resulted in fewer viable cells and more apoptotic cells. The percentage of apoptotic cells was higher in the combination treatment of cisplatin with HSD compared to the combination with DSM.

Cisplatin (Cisp) is preferred because it is eliminated through the kidneys, resulting in fewer side effects. However, like other anticancer drugs, Cisp also damages normal cells, leading to non-selective therapy [16]. Further observations regarding the co-treatment effect of HSD or DSM with Cisp on normal kidney cells showed that co-treatment HSD or DSM with Cisp reduced the number of senescent cells in normal Vero cells. Besides, it also yielded Combination Index (CI) values ranging from 14.3-85.9 of HSD and 7.43-38.99 of DSM in Vero cells, suggesting that the combined treatment was more effective as an anticancer therapy with reduced side effects. Studying the alterations in cellular physiology caused by HSD and DSM administration may uncover their specificity toward cancer cells. These findings align with prior research indicating that cell senescence predominates over apoptosis in tumor cell lines treated with chemotherapeutic drugs, each exerting distinct effects.

HSD and DSM are utilized in this study as representative synthetic natural products. They have shown a synergistic effect when combined with Cisp on cancer cells and an antagonist effect on normal cells. Our results indicated that HSD or DSM enhanced the cytotoxic effects of cisplatin, induced senescence and apoptosis in HepG2 cancer cells, and protected senescence in normal kidney cells. HSD and DSM can enhance the cytotoxic effects of Cisp on HCC cells while mitigating its adverse effects on normal kidney cells. These observed effects suggest the potential use of HSD and DSM as adjunct chemotherapeutic agents with Cisp.

Author Contribution Statement

Anif Nur Artanti: Data acquisition and analysis, analysis of the data, drafting the manuscript, revising the manuscript. Riris Istighfari Jenie: Research idea and finding funding for the research, design the research, supervising the experiment, analysis and curating the data, drafting the manuscript, revising the manuscript. Rumiyati: Supervising the experiment, revising the manuscript. Edy Meiyanto: Research idea, supervising the experiment, analysis and curating the data, revising the manuscript

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Approval

This study was part of the dissertation of Anif Nur Artanti as a student in Doctoral Program of the Faculty of Pharmacy, Universitas Gadjah Mada.

Conflict of Interest

Authors declare that there is no conflict of interest

Ethical Declaration

This study does not involve human participants or animals. Therefore, it does not require an institutional review board or ethics committee approval.

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